

LIFE HISTORY STUDIES ON THE TREMATODE FAMILY
BUCEPHALIDAE

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The order Gasterostomata Odhner is unique among the trematodes in that its members possess a ventrally located mouth in place of the usual terminal mouth, and a sac-like gut resembling the gut of rhabdocoele turbellarians instead of the usual forked intestine. The sac-like gut was observed for the first time by von Siebold (1848). The presence of the ventral mouth and rhabdocoele gut, in the absence of sufficient evidence from life histories and embryonic development, caused Odhner (1905) to place the gasterostomes in a separate order. The taxonomic position of the group has been for many years of much interest to the systematist. Many of the structural characters of the order have puzzled investigators. Is the ventral pharynx homologous with the ventral sucker of distomes? If so, is the connection of the gut to the sucker, primary or secondary? Do these structures show the possible origin of the trematodes from the free-living turbellarians? Are they primitive forms or are they degenerate distomes? Ziegler (1883) and Tennent (1906) provided an extensive survey of the literature concerning the gasterostomes. Tennent worked upon marine forms and described the life history of a gasterostome for the first time. Kelly (1899) in a statistical study of the parasites of the Unionidae found *Bucephalus* in many species of the family and widely distributed. He referred also to the unsexing of the mussel and its subsequent result in change of shell-form. Stafford (1904) described but did not figure *Gasterostomum pusillum* from the intestine of *Stizostedion vitreum* Mitch., secured from Canadian fish markets. Cooper (1915) described and figured a gasterostome from the stomach, intestine and caeca of *Micropterus dolomieu* and intestine of *Boleosoma nigrum*, from Canadian fresh waters which he referred tentatively to the species *G. pusillum* Stafford 1904. Cooper apparently did not have *G. pusillum* but from all indications the form which he described is the same as is presented in the following pages.

The Problem. The problem that I set for myself is divided into six parts: (1) to discover the various stages in the life history of a fresh-water gasterostome; (2) to organize this material into a connected story of the life history; (3) to find the miracidium and determine, if possible, its flame-cell pattern; (4) to study the details of the flame-cell pattern of the cercaria; (5) to study the development of the peculiar tail of the cercaria; and (6) to find evidence for or against the indicated relationship expressed by La Rue (1926), that the gasterostomes are related to the Strigeidae and the schistosomes.

* Contribution from the Zoological Laboratory of the University of Michigan.

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Material. Molluscs which might serve as the intermediate host for gasterostomes were gathered in large numbers from the Huron River, near Ann Arbor, Michigan, and from nearby lakes and streams. After the first season's work, attention was focused on collecting only the two species of Unionidae that had been found infected with gasterostome cercariae. The mussels collected were placed singly in fingerbowls for a few days and the individuals which gave off cercariae were retained. An abundant supply of cercariae, both in free-swimming and developmental stages, was secured from infected mussels kept in the laboratory during the winter months.

TABLE I
MOLLUSCS COLLECTED IN THE VICINITY OF ANN ARBOR, MICHIGAN

Species ¹	No. of examined specimens	No. infected with gasterostomes	Total % of infection
<i>Lymnaea stagnalis appressa</i> Say.....	238 ²	0	—
<i>Lymnaea palustris</i> Adams.....	196	0	—
<i>Planorbis trivolvis</i> Say.....	83	0	—
<i>Physa sayii</i> Tappan.....	50 ²	0	—
<i>Campeloma subsolidum</i> (Anthony).....	62	0	—
<i>Goniobasis livescens</i> (Menke).....	46	0	—
<i>Cyclonaias tuberculata</i> (Raf.).....	110 ²	0	—
<i>Elliptio dilatatus</i> (Raf.).....	2170 ²	233	9.3%
<i>Alasmidonta marginata</i> (Say).....	41	0	—
<i>Ptychobranhus fasciolar</i> (Raf.).....	87 ²	0	—
<i>Eurynia iris</i> (Lea).....	1200 ²	72	6%
<i>Ligumia fasciola</i> (Raf.).....	90	0	—
<i>Lampsilis ventricosa</i> (Barnes).....	36	0	—

¹ Identifications were made by Mina L. Winslow and William Clench of the Museum of Zoology, University of Michigan, and the names used are from "A Revised Check List of Michigan Mollusca, No. 181, Occasional Papers of the Museum of Zoology, University of Michigan. Dec. 1926."

² Other species of parasites were observed in these hosts.

Fish suspected of serving as hosts for adult gasterostomes were taken from the Huron River, near Ann Arbor, Michigan, either with hook-and-line or by seining. Wall-eyed pike, *Stizostedion vitreum* Mitchell, heavily

infected with small gasterostomes were secured from Saginaw Bay, Michigan, through the Michigan State Department of Conservation. One specimen of wall-eyed pike was obtained from Houghton Lake, Michigan. A consignment of *S. vitreum* from lake Winnipeg, Canada, also harbored many adult gasterostomes.

Uninfected bass and blue-gills used in connection with infection experiments were secured in abundance from a small glacial lake, west of Ann Arbor, known locally as the Third Sister Lake in which Unionidae do not occur.

Technique. Adult gasterostomes were secured from the pyloric caeca of members of the family Centrarchidae, from the intestines of small pike, *Esox lucius* Linnaeus and wall-eyed pike, *Stizostedion vitreum* Mitchell. Best results were obtained by removing the caeca to a watch glass containing clear water and examining one caecal pouch at a time. The instant a gasterostome was observed it was removed by means of a capillary pipette to a slide for identification under higher magnification and then quickly washed into a culture dish. Any eggs shed on the slide were stored separately from those later removed by tearing the adult with needles. For culture dishes, glass salt cellars with round-bottomed depressions were used. These allowed the eggs to concentrate in a small area and thus facilitated examination with a microscope equipped with a 3 mm. water immersion lens and 15 $\times$ ocular.

Living miracidia were stained with methylene blue and pictures were taken with a Zeiss "Phoku" Photographic Eyepiece using a 4 mm. objective.

Mature cercariae were obtained by isolation of mussels in covered finger bowls. For fixation, free-swimming cercariae were plunged from a pipette into cold Gilson's fluid which fixed the cercariae with their tails extended. Some of these cercariae were sectioned, others stained and mounted *in toto*. Developmental stages were obtained by cutting away the body wall of the mussel on one side and scraping out all the tissues in the region of the gonads into a watch glass containing 0.7% physiological saline solution. Embryos remained alive in this solution for five or six hours and could be studied under a cover-glass using high magnification. Developmental stages were also fixed in Gilson's fluid, stained with paracarmine and mounted *in toto*.

The whole bodies of several infected mussels were fixed in Bouin's fluid and sectioned at thicknesses varying from 5 to 20 microns. The stains used were Heidenhain's iron haematoxylin counter-stained with eosin or light-green, Mallory's triple stain which gave excellent results, and a modified Flemming's stain using safranin, light-green, gentian violet and orange G. This last stain proved very satisfactory.

Adult worms used for whole mounts were flattened under cover glass

pressure and fixed with Bouin's fluid drawn under the cover glass with blotting paper. After several minutes the cover glass was floated off with 70% alcohol and the worms washed into a vial. Meyer's paracarmine and Ehlich's acid haematoxylin were used as stains.

Adults to be sectioned were plunged into Gilson's fluid which fixed the specimens without collapse. The modified Flemming's stain was used with gratifying success on the sectioned material. Good results were also secured with Heidenhain's iron haematoxylin counterstained with eosin.

The locomotion of the cercariae and their penetration into fish were recorded photographically on standard motion-picture film from which prints and enlargements were made in addition to the securing of a film for projection.

***Bucephalus papillosus* sp. nov.**

Synonym: *Bucephalus pusillus* Cooper 1915

Specific diagnosis: Body cylindrical, 0.728–1.024 mm. long, 0.168–0.256 mm. broad; anterior end abruptly truncate, posterior end rounded. Skin spinous. Anterior end provided with fifteen muscular papillae protruding laterally. Anterior sucker opening ventrad. Pharynx central .040–.006 mm. in diameter; intestine a simple sac median in position. Ovary on right side below the gut. Testes two, one behind the other on the right side below the ovary. Cirrus-pouch on the left side, genital pore ventral, a short distance from the posterior end. Vitelline follicles, sixteen on each side of the body, in series extending from the region of the pharynx anteriad to the vicinity of the anterior sucker. Uterus with few coils one loop only extending anteriad above the gut. Eggs few, seldom over 300, not colored, 0.028–.040 mm. by 0.014–0.020 mm. Excretory vesicle a long simple sac; pore terminal, separate from the genital pore.

Habitat. Adult in caecal pouches of *Micropterus dolomieu* Lacepede, and *Aplites salmoides* Rafinesque, and in stomach and intestine of small specimens of *Esox lucius* Linnaeus.

Locality. Ann Arbor, Michigan, Lake St. Clair.

Type specimen no. 313 in the Parasitology Division, University of Michigan Museum of Zoology.

Description. The body is capable of great extension and contraction. Immature worms in motion show a very characteristic abruptly truncate anterior end and a rounded posterior end. A mature adult when extended to its full length, or completely relaxed, reveals an almost complete ring of fifteen muscular papillae (Figs. 12 and 13) protruding laterally from the margin of the flat terminal surface. The papillae are withdrawn when the worm is contracted. The flat terminal surface of the anterior end is circular except for a small, rounded notch (Figs. 12 and 13*n*) on the ventral margin, over the anterior sucker. The anterior sucker 0.136–0.192 mm

in length, is sub-terminal on the ventral side with the cavity extending deeply into the body. Small spines covering the entire body and the lining of the anterior sucker, are arranged in a quincunx pattern.

Large sub-cuticular glands are scattered over the body, being more numerous and conspicuous in the anterior third. Short ducts lead to the surface from these glands, often anastomosing with other ducts. The mouth, having an upper and lower lip but no oral sucker, is located midway of the body. The genital pore opens on the ventral surface a short distance from the posterior end. The excretory pore opens separately on the posterior end.

TABLE II.
SPECIES OF FISH INFECTED WITH *B. papillosus*.

Species of Fish	Locality	No. examined	No. infected	% of infection	No. of <i>B. papillosus</i> recovered
<i>Micropterus dolomieu</i>	Third Sister Lake, Ann Arbor, Mich.	75	0	0	0
" "	Lake St. Clair	40	5	12.5	15
" "	Pontiac, Mich.	6	0	0	0
" "	Huron River, Ann Arbor.	273	30	11	250
<i>Aplites salmoides</i>	"	3	3	100	70
<i>Esox lucius</i> , 10''-18''	"	17	5	29	30
" " 18''-28''	"	3	0	0	0

Digestive System. The thick-walled muscular pharynx lies so close to the mouth that a distinct prepharynx is not evident. The esophagus is short and directed obliquely forward to the anterior end of the gut, which is small, 9.096-0.208 mm. in length, oval in outline and located slightly anterior to the middle of the body. The wall of the gut consists of a single layer of large cubical cells.

Excretory System. A conspicuous excretory bladder having a black appearance in living specimens when viewed with transmitted light extends from near the base of the anterior sucker, to the posterior end of the body in the form of a sigmoid curve.

Reproductive system. The testes, nearly equal in size and 0.064-0.128 mm. in diameter, are globular in shape, and lie one behind the other on the right side below the ovary. From the left side of each testis a vas efferens extends forward uniting to form the very short vas deferens just previous to entering the seminal vesicle. This latter is enclosed in a long narrow cirrus pouch which lies in the posterior third of the body on the left side.

The seminal vesicle continues as a straight tube through the center of the cirrus pouch, and is surrounded by a mass of prostate gland and parenchyma cells. The pars prostatica is a narrow continuation of the seminal vesicle and extends as a short tube the ductus ejaculatorius at the base of the cirrus, which extends as a cylindrical protuberance into the genital sinus.

The ovary is in the middle of the body on the right side posterior and ventral to the gut. It is ovoid, 0.048–0.088 mm. in diameter. A short oviduct originates on the posterior side of the ovary and extends for a short distance as an expanded tube, which functions as a seminal receptacle. Laurer's canal is given off almost immediately from the oviduct and extends for a short distance to open on the dorsal wall of the body at the right of the median line. The oviduct then turns sharply to the left and receives the common vitelline duct just before the oviduct becomes the oötype. The vitelline follicles, sixteen on each side of the body are grouped in linear series in the second fourth of the body from the anterior end. Lateral vitelline ducts extend on the dorsal side of the body posteriad to the region between the ovary and the anterior testis, where they turn almost at right angles and unite in the dorsal median plane into a very short common vitelline duct whose function with the oviduct has been described. The oötype, short and not much expanded, is surrounded by the small Mehlis gland which consists of a group of elongated cells connected to the oötype by short ducts. The uterus, beginning in the oötype turns dorsad towards the left, ascends to the region of the posterior end of the gut, bends sharply to the right and descends to the vicinity of the junction of the lateral vitelline ducts. It then bends sharply anteriad in its course and ascends to the left and then downward, extending in almost a straight path, on the ventral side, to the genital sinus.

The egg (Fig. 1) of *B. papillosus* is ellipsoidal. Considerable size variation exists in the eggs of different specimens and sometimes within a single individual. Measurements of fifty eggs in all degrees of development, removed from the uterus of several adults are: Length .028–.040 mm., average .039 mm., width .014–.020 mm., average .017 mm. The shell is thick and colorless but has granular patches irregularly placed over the surface. The operculum is pushed completely off when the miracidium emerges. The number of eggs in a normal individual is about 300. The eggs when first deposited in the uterus have a large mass of yolk material at either end with the ovum in the center while the eggs near the genital pore contain mature miracidia ready to hatch. In young individuals the eggs were malformed and contained a dark yellow material with no sign of a developing embryo, but in older individuals no malformed eggs were noted. In order to secure clean eggs, the adults were washed repeatedly in tap water and then the eggs were secured by tearing the adults with needles. The round-bottomed salt cellars used as culture dished were then sealed

with vaselined glass covers and no additional water was added during the period of observation, which sometimes extended over a period of several weeks. As the embryo develops, the yolk granules lose color and finally are barely noticeable. The embryo contracts from the egg-shell, leaving a large space on one side, which is generally occupied by two large vacuoles. In a well developed miracidium waves of ciliary action pass from anterior to posterior at more or less regular intervals and the body squirms and changes its position slightly. If the operculum is loosened by the repeated blows from the cephalic end of the miracidium water enters and stimulates the cilia to rapid movement. The miracidium with a shiver and a shake knocks the operculum completely off and escapes into the water. This occurs with great rapidity making it difficult to analyze the steps in the process.

The mature miracidium is normally ready to hatch as soon as the egg comes in contact with water. This was made evident when eight adults were taken from the caecal pouches of a 10-inch small-mouthed bass and quickly placed in water. Within five minutes a miracidium was noticed in the dish. Two hours later eight had emerged. About eighteen hours later a number of eggs from these adults were placed on a slide, a small amount of methylene blue powder was added to the water and stirred with a needle. The cover-glass was immediately placed on the small drop and examination revealed fourteen miracidia free from their egg shells, some swimming actively about but most of them stuck by the anterior region, to the coverglass, slide or debris, but with the cilia in rapid motion.

The body of the miracidium (Fig. 2) is ellipsoidal, with an attenuate cephalic end. No anterior knob or stylet was observed. The surface of the miracidium is not ciliated. There is, however, a locomotor apparatus consisting of four plates and three pairs of jointed appendages. The plates are attached at their anterior ends to the cephalic region of the body and vibrate rapidly up and down. Each plate bears two rows of long backwardly inaligned cilia. The three pairs of single-jointed caudal appendages are attached to the posterior half of the body. One pair is dorsal, the second lateral and the third ventral in position. Each appendage has a joint about one-third of its length from the distal end. From this joint outward, each appendage bears a double row of very long cilia. The total length of the appendage, not including the cilia, approximates that of the body. In swimming the appendages are kicked backward propelling the miracidium rapidly through the water.

Wagener (1858) figured the embryo of a gasterostome within the egg-shell but the only feature that is possible of recognition is the cross-mark at the anterior end which I interpret to indicate the presence of four cephalic plates. Willemoes-Suhm (1873) illustrated the embryo of *Gasterostomum crucibulum* Rud. showing what appears to be a stylet. Wedl

(1857) showed the embryo of *Distoma campanula* Duj. 1848, a form which Wagener (1858) concluded to be synonymous with *G. fimbriatum* v. Siebold 1848. The figure clearly indicates the position of the cephalic plates, although Wedl did not recognize them as such.

Sporocysts or germ tubes containing cercariae of *B. papillosus* were found in *Elliptio dilatatus* (Rafinesque) = (*Unio gibbosus*) which is the most common mussel in the Huron River. As may be seen from Table I, about ten per cent of the specimens examined were infected. When first discovered on July 15, 1926, with the water temperature at 25°C., the quantity of sporocysts in a single mussel was very great. The tubes extended throughout every part of the body formerly occupied by the gonad tissue, and in many specimens extended into the liver and over the pericardial tissues. At this season of the year the tubes appear snow-white due to the presence of many mature cercariae. During the winter months infected specimens taken from the cold water of the river showed the sporocyst tubes clear and inconspicuous with most of the cercariae not far advanced beyond the germ ball stage. The tubes when heavily filled with all developmental stages have many constrictions, followed by swollen areas packed with cercariae. In the winter condition the tube is of a uniform diameter throughout. The sporocyst of *B. papillosus* resembles in many respects the sporocysts of *B. haimeanus*, described by Tennent (1906), from the oyster.

DEVELOPMENTAL STAGES OF THE CERCARIA

Reuss (1902, 1903), Haswell (1905), Tennent (1906), Cary (1909) and Faust (1917) sought to demonstrate that cercariae develop from parthenogenetic ova. Many investigators have described mature cercariae but very few studies have been made of the developmental stages that precede emergence from the mollusc. Several authors have figured developmental stages by means of outline drawings but only a few have considered the details of development of the excretory system and other organs of the early embryo. Looss (1894) showed in a series of drawings, the development of the main ducts of the excretory system of the cercaria of *Amphistomum subclavatum*. Ssinitzin (1911) described what he considered to be the probable development of the various types of cercariae, including certain phases in the development of the body and tail of cercaria but without considering its excretory system. In the course of my study I made observations on the development of the excretory system of *B. papillosus* from the first appearance of a pair of flame cells, the condition found in the mature cercaria, on the possible origin of the sac-like gut and its relation to the pharynx.

Specimens were obtained from the gonads of *Elliptio dilatatus* and studied alive in saline solution under moderate cover-glass pressure. Embryos under observation may be kept alive for several hours by occasionally

introducing a small drop of physiological saline solution beneath the cover-glass.

Development takes place at such a rapid rate in the early stages that it is necessary to select some character that will serve in the determination of the age of the embryo. The flame cells which may readily be seen when the embryo is in a flattened condition serve very well for this purpose. Three stages in early development may be recognized: the germ ball stage, the four flame-cell stage, and the seven flame-cell stage.

Germ Ball Stage. Very early in the development the embryo consists of a ball of loosely aggregated cells surrounded by a thin membrane. Tennent (1906) pointed out that in *B. haimeanus*: "In the seven-cell stage two of the smaller cells (found in the third division) have again divided, and two of these cells have taken a position on the surface of the irregular clump of cells. These two cells flatten, the flattened edges growing out and forming a membrane which encloses the young germ ball. It seems to form the basement membrane lying below the cuticle in the mature cercaria." The "germ-ball" stage includes the stages of growth of the embryo from early cleavage until the rudiments of the tail furcae make their appearance. In living germ-balls a conical depression is observed at the posterior end of the embryo. This depression soon deepens and extends as a narrow cavity, the primitive gut, into the center of the mass of cells. Very soon, thereafter, the anterior part of this cavity becomes enlarged and occupies the major portion of the center region of the embryo. A pair of large laterally placed cells oppose one another across this depression. The later history of these cells seems to indicate that they are the fundamentals of the two long tail furcae of the mature cercaria.

A frontal section (Fig. 4) of a slightly more advanced stage shows a well defined layer of small ectodermal cells. The primitive gut extending nearly to the anterior ectodermal layer is outlined by a regularly arranged row of cells, of the same size as the ectodermal cells. A group of larger more deeply stained cells, located to one side of the primitive gut, I interpret to be of mesodermal origin, giving rise to the musculature of the pharynx. A mass of smaller, lightly stained cells occupies the remaining space between the ectodermal layer and the walls of the primitive gut.

A pair of flame cells is visible in the upper half of living germ-balls one on the right side and the other on the left. The tail furcae are noticeable as a mass of cells on the lateral margin of the depression.

Four Flame-cell Stage. (Fig. 3) The embryo, at the stage when four flame-cells are visible, starts to elongate. The furcal buds are visible as short projections widely separated. A transverse line formed by the parallel arrangement of ectodermal cells is faintly visible midway between the region of the primitive pharynx and the furcal rudiments. This line indicates the position of the constriction later formed between the tail and the

body. The rudiment of the penetration glands is noticeable at the anterior pole of the embryo, as a group of cells arranged in a concentric fashion. The cavity of the primitive gut in the center of the embryo is observed to have several short processes, one extending anteriorly, one to the right and a third to the left. The pharynx appears as a ring of large cells midway of the body, ventral to the gut. A pair of cells is visible between the pharynx and the ectodermal wall. One cell is anterior to the other and these seemingly indicate the future lips of the mouth. An anterior and a posterior pair of flame cells are present. Excretory tubules are faintly visible connecting the anterior with the posterior cell and a dorsal cross-tubule apparently connects the right and left pairs (Fig. 3). In a few favorable specimens a tubule was observed which appeared to connect the middle of the transverse tubule with the posterior region of the gut.

Seven Flame-cell Stage. (Fig. 5) Division of the four flame cells of the previous stage does not take place simultaneously. The posterior pair divide before the anterior and the right anterior flame cell before the left, resulting in an asymmetrical pattern which is maintained even in the adult.

The embryo is globular in form with the two rudiments of the tail furcae more distinct and relatively not so widely separated as in the preceding stage.

A few elongated cells can be observed extending fan-like over the posterior dorsal surface. For the sake of convenience these cells are designated in the following stages as overgrowth cells. The constriction serving to separate tail from body observed in the preceding stage is noticeably deeper. Lateral excretory tubules are now visible extending from the anterior flame cells to the tips of the tail-buds, with side branches extending to the other flame cells. The tail-stem in the following stages serves as an indicator of age since the flame cells through rapid division have so increased in number that it becomes difficult to be sure of an accurate count. For convenience three stages are recognized, namely: the rudimentary furcae stage, the crossed furcae stage, and the spread furcae stage.

The Rudimentary Furcae Stage. (Fig. 6) The constriction noted in preceding stages, together with slight constrictions at the base of the rudiments of the furcae, has now definitely set apart a tail-stem between the body proper and the rudimentary furcae. In this and in the following described stages the great similarity between the tails of the cercariae of gasterostomes, Strigeidae and Schistosomes is clearly apparent.

The tail has short, rounded furcae converging inward towards the longitudinal axis of the body. The penetration organ is now well formed and plainly visible as a sharply defined mass of cells with a basement membrane. The nervous system is visible in living specimens stained heavily with methylene blue, the ganglia and lateral cords taking on a greenish hue. The gut is usually visible as a large thin walled sac, extending posteriorly

from near the brain to the region of the genital pore. The latter surrounded by a small ring of cells is plainly visible on the ventral surface near the posterior end of the body.

In oblique light, conspicuous surface markings are observed which cannot now be interpreted. These markings appear as half-round grooves originating at two conspicuous depressions located a short distance apart on the dorsal surface on either side of the median line, just posterior to the middle of the body. One pair of grooves curve posteriorly to the lateral margin, another pair of shallower grooves extends anteriorly to the region of the most anterior flame cell. The two large depressions mentioned above are associated with two large, inner cavities adjacent to the dorsal wall from which a pair of large tubules curve posteriorly, entering a new invagination which has appeared on the median dorsal wall at the junction of the body and midpiece of the tail. These two large cavities may be analogous to structures observed by Faust (1917) in *Cercaria gracillima* a form which he suggested should be referred to the schistosomes.

The "accessory" bladders which I have observed in this stage of development of *B. papillosus*, appear to be the locus of the changing arrangement of excretory tubules. Just as the primary arrangement of four flame cells with transverse connecting tubule and a connection with the primitive gut gave way to a secondary, paired lateral system extending to the tips of the furcae, in this third change, accessory bladders are formed having tubules extending to the dorsal surface at the posterior end of the body. The lateral tubules are beginning to converge and are also in connection with the so-called accessory bladders.

It is conceivable that in this stage we have the clue to the excretory system of the mature cercaria. The invagination on the dorsal wall at the posterior end of the body into which enter the two excretory tubules from the accessory bladders possibly represents the invagination cavity described by Ssinitzin (1911) which he says develops into the "true bladder (vesica vera)."

The Crossed Furcae Stage. (Fig. 7) The outstanding character of this stage is the presence of the inward-curved, crossed tail furcae. Growth of the furcae has apparently taken place faster along the outer margin, thereby forcing the furcae to bend inward, consequently causing the excretory pores of the lateral branches, formerly terminal in position, to lie on the inner margin of the furcae, removed from the tips. The lateral tubules have become fused at the junction of the body with the tail-stem. An accessory bladder has formed on each lateral tubule midway in its course through the tail-stem. New tubules extend from each accessory bladder; one branch opening into the posterior end of the primitive gut on the posterior end of the tail-stem, a second extends laterally to the margin of the tail-stem near its junction with the furcae, while a third opens on the lateral margin

of the tail-stem near its junction with the body. These openings, together with the original opening in the furcae, provide four exits to the lateral excretory system. As only one excretory pore may survive in the mature cercariae this branched arrangement provides a scheme for all the various patterns of the excretory tubules found in the tails of forked-tailed cercariae. This suggests the possibility that the position of the excretory pore may be a factor indicative of relationship.

In the gasterostome larva, as may be observed in embryos in the next stage, the pore that persists is the anterior one opening near the lateral junction of the body with the tail-stem.

The Spread Furcae Stage. (Fig. 8). Growth of the inner cells of the short tail furcae, has speeded up, causing the furcae to diverge, forming an angle of 90° between them. Within the body the lateral excretory tubules, which have drawn inward to fuse in the median plane, fuse secondarily with the tubules connecting the accessory bladder with the ectodermal invagination cavity. The fused lateral tubule passes into the tail-stem, extending for about one-fourth the diameter of the tail-stem as a short, straight posteriorly directed tube. It then branches to the right and left, enclosing an angle of 90° . After a short course each branch turns antieriad and lateral and opens on the margin of the tail-stem, adjacent to the body.

The overgrowth cells have spread over a large part of the posterior surface of the tail-stem and mark the site of the two characteristic enlargements seen in this region of the mature cercaria. In later stages of development the fused excretory tubule within the body, develops into a conspicuous elongated excretory bladder extending to the vicinity of the anterior sucker.

The observations above recorded seem to indicate that in this species of *Bucephalus* there is a fusion of the two types of bladder formation discussed by Ssinitzin (1911).

The mature cercaria (Fig. 9) of *B. papillosus* is very similar in appearance to the cercaria of *B. haimeanus* Lacaze-Duthiers, as described by Tennent (1906) and to *C. hydriformis* described by Ssinitzin (1909b).

TABLE IV.
MEASUREMENTS IN MILLIMETERS OF SEVEN, MODERATELY EXTENDED LIVING CERCARIAE OF
B. papillosus.

Body, length	0.10-0.112	average	0.11
Body, width	0.04-0.08	"	0.07
Tail-stem, length	0.012-0.015	"	0.13
Tail-stem, width	0.112-0.150	"	0.123
Penetration organ, length	0.016-0.018	"	0.0175
Furcae, extended, about 4 mm.			

The body of living *B. papillosus* is opaque, appearing snowy-white against a dark background. The lance-shaped body tapers more sharply

from the region of the pharynx toward the anterior end than toward the posterior. The entire surface of the body is evenly covered with fine spines, arranged in quincunx pattern. The spines are larger and more noticeable in the anterior third of the body. The penetration organ (ao) is pyriform with the small end forward. This small end is invertible, each lip being covered with rows of large cuticular spines, pointing outward when the lips are withdrawn. No stylet is present in this cercaria. The posterior portion of the penetration organ is composed of two groups of penetration glands; a small anterior group of small granular cells and a closely associated posterior group of many large, finely granular cells. The gland cells are connected through short separate ducts to the mid-region of the invertible spiny area. When the anterior end of the cercaria is in contact with the tissue of a fish, the penetration organ is protruded, forming a spiny knob-like end and bringing the openings of the ducts of the penetration glands into contact with the tissue of the host. The invertible spiny area is thrust in and out against the host tissue until a hole for entry is made aided by the secretions from the penetration glands. A pair of large sub-cuticular glands (sgl) are located laterally on a transverse line with the middle of the penetration organ. Ducts leading anteriorly were noted, but I was unable to verify the position of the external openings.

Large unicellular sub-cuticular gland cells are visible through the outer integument, especially when the cercaria is about to disintegrate under cover-glass pressure. These gland cells are uniformly distributed over the body and are apparently identical with the sub-cuticular glands visible in the adult.

The pharynx is located slightly posterior to the middle of the body. In living material the gut is very difficult to observe, since it is present as a many-pouched, thin walled sac. In specimens that have absorbed water, the gut is expanded into a large globular sac occupying the major portion of the body.

An accurate count of the flame cells has not been made in the mature cercaria of *B. papillosus*. The flame cells are small and numerous; the capillary tubules arising in them difficult to follow. Many counts were made, sufficient to indicate that there are a greater number of flame cells on the left side than on the right. The capillary tubules discharge into an anterior collecting tubule and a posterior collecting tubule on each side of the body. These collecting tubules join the main lateral collecting tubes at a point approximately midway between the pharynx and the posterior end of the body. From the point of union these collecting tubules pass posteriad for about one-half of the remaining distance to the posterior end of the body and then turn nearly at right angles and enter the excretory vesicle (ev) through its lateral surface. This system thus provides an anterior and a posterior group of flame cells.

A genital pore (gp) was observed on the median ventral surface near the posterior end of the body. The reproductive organs are not noticeable in living specimens but are seen in whole mounts as a mass of deeply stained cells, dorsal to the excretory vesicle, midway between the pharynx and the posterior end of the body.

When the cercaria is not extended, the anterior surface of the tail-stem forms a collar about the basal portion of the body proper. From the margin of the posterior end of the body a conical sheet of muscle fibers (mf) extends through the tail-stem to the center of the posterior wall. This mass of muscle fibers is apparently responsible for the shallow depression which is observable on the posterior surface of the tail-stem. Two pad-like masses of overgrowth cells (og) whose origin and growth were described in the developmental stages of the cercaria, extend over the basal part of the tail-stem from the posterior surface depression to the beginning of the furcae.

The caudal excretory tube (cet) within the tail-stem has the appearance noted in the description of developing cercariae at the spread furcae stage. The ascending portion (ab) of the branches of the caudal excretory tube lie adjacent to the conical sheet of muscle fibers. The two excretory pores (rep, lep) are located laterally on the tail-stem close to its junction with the body. At each contraction of the excretory vesicle liquids were observed to pass into the caudal excretory tube (cet) and by way of the bifurcations, leave the tail-stem through the lateral pores. A layer of sphincter muscles (sm) surrounds the outlet of the excretory vesicle and prevents return of liquids forced into the caudal excretory tube. The above differs markedly from the observations of Tennent (1906:659) who stated in the description of the "Water Vascular System" of *B. haimeanus*; "upon watching it for a time it may be seen to contract quickly, when its contents are forced back into the middle piece of the tail and thence into the lumen of the tails. The excretory pore on the ventral surface may be seen in the living animal. Sections reveal a somewhat strange arrangement of outlets. From the duct passing through the contractile sac to the cavity of the tail, two short canals are given off, one which passes to the dorsal surface, where it opens through a very fine pore, and one to the ventral surface at the junction of the body with the median portion of the tail (Figs. 24 and 25). When I first noticed this peculiar condition I attributed it to some abnormality, but an examination of several series convinced me that it is the usual arrangement."

The furcae are covered with a thin homogeneous cuticle. A reticulated layer of muscle fibers encloses a lumen filled with a liquid in which float small droplets of a light canary-yellow substance which has the optical properties of oil. Tests with Sudan III and osmic acid proved that these are oil droplets. Large cells are attached irregularly to the wall of the cavi-

ties. The furcae are circular in cross-section and in the extended condition have almost a uniform diameter from base to tip.

The mature cercariae of *B. papillosus* after emergence from the sporocysts leave the gonads of the mussel through the genital opening and pass out through the exhalant siphon. No birth pore has been recorded in the literature for this type of branched sporocyst. Although I have not observed cercariae emerging from the sporocyst tubes, I am of the opinion that they are able to break out through the wall of the sporocyst into the lumen of the gonads and work their way to the genital pore. When the cercaria finds itself free from the siphonal openings of the mussel, it struggles against the excurrent of water and soon extends its furcae straight out at right angles to the plane of the body (Fig. 14) measuring about 10 mm. from tip to tip. This form is essentially a "floater" and appears never to use its tail as a swimming organ. The cercaria floats along in the current until it becomes entangled on the fin of a fish or meets some obstruction. In the quiet water of a finger bowl the cercaria drops to the bottom as soon as it leaves the influence of the current of water from the exhalant siphon. It is unable to rise, but extends and contracts its furcae for some time. The tails of some specimens are shed and the body proper moves about with a leech-like movement for two or three hours.

In a previous paper (Woodhead, 1927) I reported the penetration and encystment of the cercaria of *B. papillosus*. A small (two-inch) rock bass, *Ambloplites rupestris* was placed in a finger bowl containing a fresh-water mussel (*Elliptio dilatatus*) which was rapidly shedding active cercariae. The cercariae do not actively seek the fish, but depend upon water currents to bring them in contact with a fish, into which they may penetrate. As the fish moved about cercariae became entangled on the margin of the fins, especially the dorsal and caudal. Active movements of the fish resulted in many of the cercariae being cast off. After a few minutes the fish was transferred to a watch glass and the movements of the cercariae observed under a binocular microscope. At first the furcae of the tail aided the larva in maintaining its anterior sucker in contact with the fin. The furcae appeared to exude a sticky substance and rolled up into a confused coiled mass, as the body began to penetrate. Within five minutes the body had become separated from the tail and had entered the tissue between the fin rays. The course of the cercaria was well defined by the bright pink color of the blood corpuscles which gathered in the wound. By means of a worm-like movement, the cercaria, within thirty minutes of its entanglement of the fin, completed its excursion within the host and then squirming around in one spot, enclosed itself in a thin walled, transparent cyst.

Encystment usually takes place at the base of the fin rays, under the last few rows of pigmented scales. Five hours after formation of the cyst active movements of the penetrated tailless cercaria were observed. Within

twenty-four hours the characteristic cyst was complete. The cercaria had begun the process of metamorphosis and the large excretory vesicle had become dark when viewed by transmitted light. My observations and experiments have not shown the origin of the cyst wall. That it is of parasite origin appears likely from the rapidity and method of formation. One layer only was observed in the many cysts examined. Investigations on the chemical nature of this cyst wall and its relation to the sub-cuticular glands might be worth while. These glands termed cystogenous glands by many writers, appear to carry over into the mature adult condition where they undoubtedly have a different function.

For the completion of the life cycle of the parasite the fish carrying the cysts must be eaten by a larger fish. When this occurs the cyst wall is digested away by the digestive juices of the host and the young parasite is freed. If the fish is the proper host the parasite may become established in the pyloric caeca.

DISCUSSION

Cooper (1915) described an adult trematode as *Gasterostomum pusillum* Stafford 1904 from *Micropterus dolomieu* and *Boleosoma nigrum* giving measurements and remarked that "Although these do not closely agree with those of Stafford, I am inclined to refer the worm to this species, at least tentatively, owing to the fact that most of them are immature, only a few of them being found to contain eggs. The capacious anterior sucker, which occupies the whole of the diameter of the anterior end, is truncate forward and flattened ventrally, the two surfaces meeting at right angles. Its oral sucker is situated in the mid-ventral surface and leads by a short, forwardly directed oesophagus into a comparatively small, ellipsoidal intestine which extends in front and behind the mouth."

Cooper's description agrees well with my observations on *Bucephalus papillosus*. He made no mention, however, of papillae, and his figure is not clear on this point. Ward (1918:379) describing this form adds the statement: "Anterior end bears a large sucker with ventral sac and small muscular papillae at lateral angles." In many of my specimens, mounted *in toto*, only certain of the papillae are visible, giving an impression of "papillae at lateral angles." The entire series of fifteen papillae, however, is best observed in living material.

Since the type host of *G. pusillum* Stafford is *S. vitreum* in which fish I find a form agreeing closely with the original description (Stafford 1904: 494) and differing greatly from *B. papillosus*, I am of the opinion that the specimens assigned tentatively to *G. pusillum* by Cooper are referable to *B. papillosus*. *B. pusillus* Stafford 1904 will be further described and figured in another paper dealing with life history studies of the gasterostomes.

SUMMARY

Bucephalus papillosus a gasterostomatous trematode from fresh water bass is described and figured in all stages of its life history.

The miracidium of a gasterostome is described in detail for the first time. The miracidium is equipped with four cephalic plates covered with long cilia and three pairs of posterior jointed appendages, also bearing very long cilia.

The embryology of the cercaria is briefly indicated.

The penetration of the cercaria into small fish is described.

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EXPLANATION OF PLATE

Drawing of the adult was made with the camera lucida; others free hand, some with the aid of photomicrographs.

Abbreviations used:

a	appendage
ab	ascending branch
ao	anterior organ
as	anterior sucker
bp	cephalic plate
cet	caudal excretory tube
ev	excretory vesicle
gp	genital pore
lep	left excretory pore
mf	muscle fibers
mgl	Mehlis gland
n	notch on anterior surface
o	operculum
og	overgrowth cells
p	proboscis
rep	right excretory pore
sgl	sub-cuticular gland
sm	sphincter muscle
ts	tail-stem

PLATE XXXI

All figures concern *B. papillosus*.

- Fig. 1. Egg from proximal region of uterus.
- Fig. 2. Lateral view of miracidium.
- Fig. 3. Diagrammatic ventral view of germ ball at the Four Flame-cell Stage.
- Fig. 4. Sagittal section of early germ ball.
- Fig. 5. Embryo of cercaria at the Seven Flame-cell Stage.
- Fig. 6. Embryo at the Early Furcae Stage.
- Fig. 7. Embryo at the Middle Furcae Stage.
- Fig. 8. Embryo at the Late Furcae Stage.
- Fig. 9. Mature cercaria, furcae have not been shown, for the sake of clearness.
- Fig. 10. Penetration organ protruded.
- Fig. 11. Sporocysts, showing nodular appearance.
- Fig. 12. Anterior surface, showing the 15 papillae.
- Fig. 13. Ventral view of adult.
- Fig. 14. Position assumed by cercaria in a current of water.
- Fig. 15. The same as Fig. 13, only much reduced to show appearance when extended.

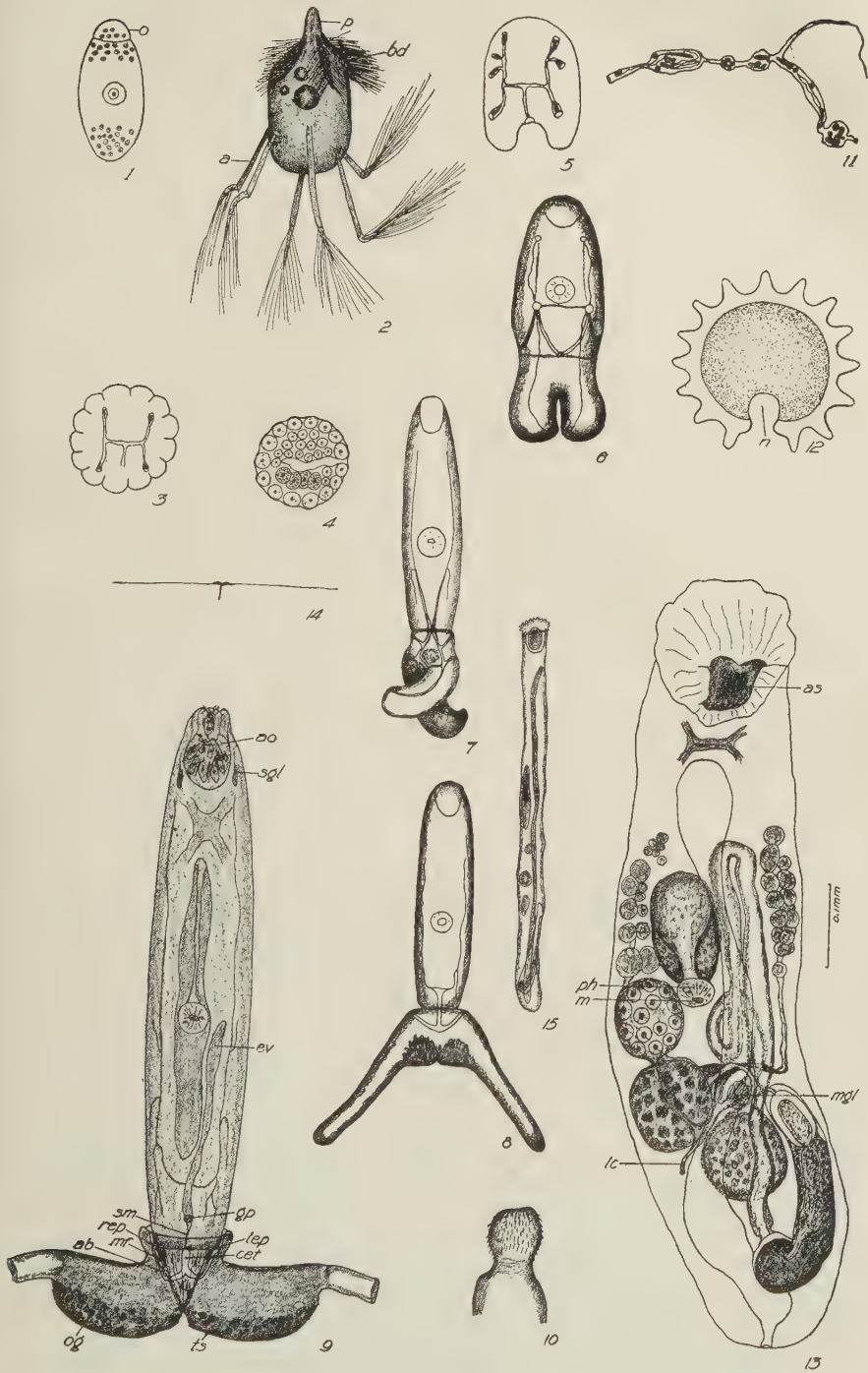


PLATE XXXI

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LIFE HISTORY STUDIES ON THE TREMATODE FAMILY BUCEPHALIDAE. No. II.*

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Early in the fall of 1926 while searching for gasterostome cercariae, two specimens of the rainbow clam, *Eurynia iris*, were found harboring a species of cercaria of very striking appearance and undoubtedly a gasterostome. As the cercaria of *B. papillosus*, Woodhead (1929), had been located in *Elliptio dilatatus*, this new find led to the belief that two species of gasterostomes were present in the Huron River, near Ann Arbor. The following summer, a large bed of rainbow clams was located and a more critical examination of adult forms was made. Late in September, 1927, an adult, bearing extensible fimbriae, was noticed for the first time. Subsequently, experiments were carried out to determine the respective life cycles. The following paper presents the life cycle of the second form, the first having been described in a previous paper (Woodhead, 1929).

Bucephalus elegans, sp. nov.

Specific Diagnosis: Body form cylindrical, rounded posteriorly, truncate anteriorly, 0.658 by 0.192 mm. The terminal anterior surface is flat or slightly convex and bears seven extensible head appendages in the form of fimbriae. The anterior sucker is large, 0.176 by 0.156 mm. spheroidal in outline, opening ventrad. Body covered with conspicuous spines. Mouth ventral, in the middle of the body. Genital pore ventral, near the posterior end of the body. Excretory pore terminal, at the posterior end. Pharynx large, 0.061 mm. in diameter, opening into an antieriad directed esophagus which enters an oval sac-like gut at its anterior end. The two testes are in line, one behind the other below the ovary. The anterior testes is the larger, measuring 0.096 by 0.084 mm. The posterior testis averages 0.084 mm. in diameter. The cirrus pouch is about one-third the length of the body, lying on the left side near the posterior end. The ovary is located adjacent to the gut on the right side antieriad to the anterior testis and averages .064

* Contribution from the Zoological Laboratory of the University of Michigan.

mm. in diameter. Laurer's canal is present. Eggs, about 200 in number, average 0.048 by 0.021 mm. when ready to be discharged. Excretory bladder a long curved sac, extending anteriorly to the anterior sucker.

Habitat. The adult (Fig. 22) of *Bucephalus elegans* is normally found only in *Ambloplites rupestris* (Rafinesque) the red-eye or rock-bass, an abundant fish in the Huron River, near Ann Arbor, Michigan. An examination of the caecal pouches of 350 rock-bass of all ages and from many different points along the Huron River showed that 210 individuals, or 60% were infected with *B. elegans*. The number of parasites recovered from each fish was seldom more than three.

Description. The form of the body, in relaxed condition, is cylindrical, rounded posteriorly, truncate anteriorly. The terminal anterior surface is flat or slightly convex, depending upon the state of contraction of the head appendages. A large notch (Fig. 18n) on the ventral margin of the anterior surface is observed in relaxed individuals. The head appendages (Figs. 17-19) seven in number, are located on the margin of the anterior surface. These head appendages, in the nature of fimbriae, appear first as short stalks with knob-like ends (Fig. 17). Later a small distal and a larger proximal protuberance appear on the underside of the fimbriae (Fig. 19) near its base. Complete extension then produces long, semi-rigid, downward-curving fimbriae with two finger-like processes near the base of each, the proximal process appearing twice as long as the distal. My observations on living specimens of *B. elegans* revealed the presence of a central cavity extending the length of the extended fimbriae and occupying about one-third of its diameter.

Wagener (1858) in a figure of an "old specimen" of *G. fimbriatum* v. Siebold 1848, showed five partly extended fimbriae. Among the many older individuals of *B. elegans* which I have examined, only one living specimen showed the fimbriae completely extended (Fig. 19). Diesing (1858) in a systematic paper created a new genus *Gasterostomum* on the basis of the complex and peculiar structure of the anterior end of *G. fimbriatum* as described by Wagener (1857). Ziegler (1883:549) pointed out what he considered the error in Wagener's interpretation of the anterior end of *G. fimbriatum* and was of the opinion that the "fimbriae" were caused by the contraction of the radial fibers of the anterior sucker.

The large spheroidal anterior sucker (Fig. 22, ao) extends from the ventral surface nearly to the dorsal surface, occupying most of the space at the anterior end of the body. It is composed of a mass of unicellular glands and radial muscle fibers, enclosed in a basement membrane. These muscle fibers are arranged in fourteen longitudinal layers which extend from the dorsal to the ventral wall of the sucker. The gland cells are located in the central region of the sucker, arranged in seven areas each area bounded on either side by one of the layers of muscle fibers. The layers of muscle fibers between

adjacent glandular areas are separated by a thin layer of parenchyma. The gland cells, of two distinct sizes, pour their secretions into a pair of ventrally located ducts which lead to the anterior surface of the body. The larger cells, fewer in number, are located in the areas between the muscle fibers and are connected with the ducts by short secondary tubules. The smaller, more numerous cells are sessile on the ducts. Large spaces, surrounded by parenchyma, are located in each of the seven areas between the muscle fibers and dorsad to the glands. These dorsal spaces are associated with the seven extensible fimbriae. The basement membrane (Fig. 22, bm), of the anterior sucker, enters into the formation of the extensible fimbriae. When muscle fibers within the sucker contract, thus reducing the size of the spaces lying between them, a liquid within the spaces is forced against certain places on the anterior portion of the basement membrane which is forced outward from the body along with the cuticle and subcuticular muscle layers. This part of the sucker which is forced outward assumes a definite form, the fimbriae. Ziegler (1883: Fig. 19) in a cross-section of the anterior region of *G. fimbriatum*, indicated six groups of these muscle fibers which by their contractions, he thought, caused the parenchyma cells and the muscle layers of the body wall between each group to be extended into a peg-shaped projection. My observations therefore tend to confirm Ziegler's conclusions as to the manner of formation of the fimbriae.

The body is covered with a thin cuticle in which are embedded flat, blunt-pointed posteriad directed spines (Fig. 23) inserted acutely on a transversely elongate basal plate. The spines are more prominent in the anterior region and cover the entire lining of the anterior sucker, indicating that the sucker is formed as a depression on the ventral surface. Elongated subcuticular unicellular glands are uniformly distributed over the surface of the body beneath the cuticle and body-wall muscles. Each cell is provided with a short duct leading to the surface, or joined to other short ducts and thence to the surface. Very little is known concerning the function of these glands. I hazard the suggestion that they may secrete a substance, perhaps mucus, that serves to protect the parasite from harmful substances, comparable to the function of mucus secreted by a fish or frog.

The gut of *B. elegans* is more nearly oval than in *B. papillosus* previously described (Woodhead, 1929) and is relatively larger, measuring 0.112 to 0.160 mm. in length. The rest of the anatomy of the adult *B. elegans* is so similar to *B. papillosus* that further description is unnecessary.

B. elegans has the largest egg of the three species of gasterostome thus far investigated. Eggs of older individuals were fewer in number and larger than eggs of younger specimens.

A mature worm secured from an experimentally infected blue-gill, *Lepomis pallidus*, contained only 150 eggs. All were of a very uniform size and

none were malformed. Discharged eggs of this individual measured 0.046 to 0.048 mm. by about 0.021 mm. The number of eggs in other individuals ranged from 50 to 250, the usual number being in the neighborhood of 200. Measurements of over 100 discharged eggs from different individuals showed a length of 0.040-0.050 mm., average 0.047 mm., and a width of 0.018 to 0.022 mm., average 0.021 mm. The size of the egg of *B. elegans* is favorable for the study of the developing embryo, but here again as with the embryo of *B. papillosus*, the presence of the ciliated appendages prevented the observation of the flame-cells. However, certain additional structures were observed in this embryo that were not apparently present in the embryo of the latter species. Two lateral anterior gland cells were observed in a mature embryo nearly ready to emerge. While within the egg shell, the skeletal rods of the embryo, because of their length, must be folded back over the body and together with the long cephalic plates present a puzzling appearance to one who has not observed the free miracidium (Fig. 2). Embryos forced from the egg by cover-glass pressure were unsuitable for study other than for observations on the structure of the appendages. A large part of the observations on the egg and miracidium which are here recorded, were made possible through the use of a binocular microscope equipped with 15x oculars and a 3 mm. water immersion objective.

Eggs secured from mature adults were stored in glass salt-cellars having round-bottomed depressions. Tap-water was used as the medium, none being added after the initial small amount. The dishes were kept covered with small squares of glass and sealed with vaseline. Eggs were observed daily until movements of the embryo were no longer visible; the longest period of observation lasting three weeks. Eggs that showed well formed embryos, hatched in from 5 minutes to 12 days. The younger embryos, representing about 50% of the number in the uterus at one time, always failed to reach maturity and only the most mature embryos, about 5%, succeeded in emerging from the egg even though many appeared capable of doing so and showed squirming movements for many days.

The miracidium (Fig. 4) of *B. elegans* resembles in part the miracidium of *B. papillosus*. The body is pear-shaped, with the cephalic end more extended than in *B. papillosus*. The four large cephalic plates are attached as in the latter form but are relatively three times longer, extending to the middle of the body. Each cephalic plate consists of a firm narrow plate, terminating in a squared or blunt-pointed posterior end. Absorption of water causes the cells of the miracidium to swell and thereby made it evident that a single basal cell is concerned in the formation of each plate. A long, conspicuous, sharp-pointed stylet (Fig. 4, a) is present which is capable of being thrust in and out. Two dorsal, sub-cuticular, skeletal rods (sr), originating close to the point of attachment of the cephalic plates, extend backwards to the posterior

end of the body, where they diverge slightly and project free as a pair of skeletal appendages (ca) each bearing two side branches, which originate close to the body and diverge at an angle of about 60° from the main stem. The four cephalic plates and the skeletal rods bear long, uniformly distributed cilia. These plates and appendages vibrate rapidly and propel the miracidium very swiftly and smoothly through the water.

Many miracidia under observation hatched in an apparently normal way, when stimulated by light from the sub-stage lamp. Emergence is preceded by a squirming movement and then by a fluttering of the appendages. The cilia begin to beat and the miracidium, knocking the operculum completely out of the way, travels rapidly through the water for a few moments. It becomes stationary the moment any obstruction is encountered. Some were observed attached to the water-film, others attached to the bottom of the dish. The appendages vibrate rapidly for a few minutes after attachment. No miracidium was observed to resume locomotion after becoming attached. A miracidium was observed attempting to emerge from the egg. The stylet was repeatedly thrust against the operculum, but in this instance without avail. Death followed the attempt, in about ten minutes. This observation suggested that the stylet may serve, in this particular species, as an aid to emergence. Normally hatched miracidia did not readily absorb water. Hours after emergence dead miracidia were apparently disintegrating without much cellular swelling.

The flame cells were not observed although many living miracidia were examined with this end in view. The presence of cephalic plates and skeletal rods covered with long cilia renders the observation of flame cells very difficult. Judging from the size of the flame cells in the cercaria, the flame cells of the miracidium must be minute.

A photomicrograph of a recently hatched miracidium served as the basis of the following measurements. The empty egg shell from which the miracidium had emerged, was estimated to be that of a large egg, which would therefore have a total length of about 0.048 mm. Based on this assumption the length of the following structures expressed in millimeters were: body of miracidium 0.049, stylet 0.014, cephalic plate 0.024, skeletal rod 0.039, branch of skeletal rod 0.016, distal end of skeletal rod from branches 0.013, cilia on the cephalic plates 0.012, cilia on the skeletal rods 0.013.

I have made no experiments or observations on the entrance of the miracidium into the molluscan host. Tennent (1909) injected a mixture of water and feces containing ripe eggs of *G. fimbriatum*, between the valves of uninfected oysters and later recovered developing sporocysts. He did not observe the hatching of the egg or the entrance of the miracidium into the gonads of the oyster. Unfortunately Tennent (1906) failed to state the location of the youngest sporocyst that he had observed.

A small mussel, *Eurynia iris* (Lea) (*Lampsilis iris*), is the first intermediate host of *B. elegans*. About six per cent are infected. This mussel occurs in the Huron River in shallow water. A very extensive bed of these mussels was located about four miles above Ann Arbor from which in October, 1927, about 1,200 specimens were obtained. Thirty-five infected specimens were secured through isolation in finger bowls. Later in the winter from this same collection about forty more infected specimens were secured, by opening mussels and examining for sporocysts, increasing the incidence of infection to about 6%.

The gonad is the organ usually occupied by sporocysts and, indeed, in many mussels which I have examined, it is the only structure occupied by them. Cross-section of a lightly infected mussel showed the gonad to be producing eggs over about 75% of its area. Other specimens had the gonad entirely replaced with sporocyst tubules. The sporocysts of *B. elegans*, found in the gonads of a mussel, *E. iris*, that had been kept in an aquarium all winter, were empty of developing cercariae. They were translucent and about the color of the gonad tissue. Snowy-white tubes well filled with all developmental stages of cercariae, surrounded the adductor muscles, covered the tissue about the pericardial chamber and extended through the tissue of the gills. I believe that the empty, brownish tubes were those that had shed their cercariae during the previous season and were now old and spent but still alive; while the snowy-white, well filled tubes that had developed from the older portion, because of the warm water of the aquarium, corresponds to the second season's period of productivity. The sporocyst of *B. elegans* differs markedly from the sporocysts of *B. papillosus*. The knotty appearance of well filled tubes of the latter species is lacking; the germ tube of the present species being nearly uniform in diameter throughout its length. At irregular intervals and from all sides, new branches are given off at right angles to the older stem. These branches are small in diameter in early stages and often extend for long distances as very fine threads. Tennent (1906) concluded from observations on the sporocysts of *B. haimeanus* that these branches are parts of the body of the sporocyst, originally developed from a single miracidium. Ssinitzin (1911) stated that he was unable to isolate a single tube from the mass of branched sporocysts of a similar form. He also states that sporocysts of this long branching type produce cercariae directly without the interposition of a second generation of sporocysts.

The development of the cercaria is similar to that of *B. papillosus* (Woodhead, 1929) except for the type of overgrowth over the posterior surface of the tail-stem. The tail-stem becomes oval in shape and the overgrowth cells form a firm covering over the posterior half. The cells are larger posteriad and gradually reduce in size until they merge into the clear region of the anteriad half.

At irregular intervals during the period of shedding, cercariae are expelled from the exhalent siphon of the mussel. Temperature is a factor influencing the period of shedding, the optimum being 17-25 degrees.

The spindle-shaped body of the mature cercaria is joined somewhat broadly to the tail-stem. The tail-stem in this species is decidedly different than that observed in *B. papillosus*. It is more or less spheroidal in shape, with the anterior and posterior surfaces somewhat flattened. Two opaque furcae, capable of great extension are inserted on the antero-lateral surface of the tail-stem.

TABLE I
MEASUREMENTS OF TEN LIVING CERCARIAE EXPRESSED IN MILLIMETERS

Length of body, contracted	0.270-0.320, average 0.298
Length of body, expanded	0.320-0.400, average 0.366
Width of body	0.080-0.104, average 0.090
Length of tail-stem	0.112-0.160, average 0.133
Width of tail-stem	0.192-0.232, average 0.216
Length of furca, contracted	0.400-0.480, average 0.440
Width of furca, contracted	0.096-0.128, average 0.111
Length of furca, extended	1.464-1.647, average 1.561

The body of the cercaria is covered with a thin cuticle, in which are imbedded short, flat, blunt-pointed spines that diminish in size from the anterior region backwards. Underneath the cuticle is a layer of very large epidermal cells which gives the outline of the body a crenulated appearance.

The anterior end of the cercaria is constructed on the same plan as previously described for the cercaria of *B. papillosus*. The penetration organ is pyriform, with the invertible portion five lobed and heavily spined. Cercariae, under coverglass pressure, were observed to exude a stream of viscid material from the penetration glands. In living cercaria treated with neutral red the large posterior penetration glands turn bright pink and the body a deep yellow. From this color change I conclude that the posterior penetration glands are acid and the body basic in character.

The pharynx is located midway of the length of the body. Sections indicate that the gut in most cases is temporarily pouched and occupies a large part of the body. In living, flattened specimens the contractions of the body also reveal this very large, many pouched gut. The gut is quite variable in size, appearing in some specimens as a very small sac with a compact single layer of cells, while in others it is large and thin-walled. Small brownish concretions are sometimes visible in the gut. These are forced from one end to the other during movements of the body.

The excretory vesicle extending from near the end of the body to about the middle line of the pharynx is easily recognized. The flame cell pattern of this cercaria is reducible to an anterior and a posterior group (Fig. 15). On

the right side 34 flame cells were observed and on the left 33, making a total of 67.

The genital pore is ventral near the posterior end of the body. The reproductive organs however are very difficult to observe in the living specimens but the fundaments of the organs are visible in individuals stained with nuclear stains.

The tail-stem is spheroidal, with the anterior and posterior surface somewhat flattened. The body proper is attached to the anterior median surface by a tubular mass of muscle fibers, which extends through the center of the tail-stem to its base, where it spreads out like a fan and is inserted on the posterior wall of the tail-stem. This fan-shaped insertion of muscle fibers produces a shallow depression on the posterior median surface of the tail-stem. The lumen of the tail-stem is filled with a clear liquid in which was observed small pale-yellow droplets, probably an oil. A narrow cord of muscle fibers extends over the lateral surface of the tail-stem from the insertion of the body to the proximal muscular region of the furcae. The two lateral excretory pores of the caudal excretory tube were observed in this cord of muscle fibers a short distance from the insertion of the body. Contraction of the tube of muscle fibers draws the body deep into the tail-stem and also causes the withdrawal of the proximal part of the furcae, which are thereby brought near to the body and serve as a form of protection for the body. The posterior half of the tail-stem is covered with large irregularly shaped overgrowth cells, that apparently lend stiffness to this region. The half facing anteriorly is composed of a thin, transparent layer of tissue.

The furcae of this species are exceptionally conspicuous. They are capable of great extension and present an opaquely white appearance making the cercariae easily visible with the unaided eye. The furcae originate on the anterior surface of the tail-stem. The proximal portion of the furcae is conical in shape and is covered with a clear, transparent tissue. This region is partially retracted within the tail-stem in all stages of swimming except during the period of greatest extension of the furcae. A mesh-like layer of sub-cuticular muscle fibers extends from the clear region at the base of each furcae to its tip. Sections of the furcae are circular in outline and show a central lumen surrounded by sub-cuticular parenchymatous tissue. In living specimens this lumen is filled with a clear liquid containing various sized granules. The cercariae shed from a certain mussel over a period of one week, were peculiar in that the central lumen of the furcae were well filled with large definitely outlined, seed-like bodies. When treated with neutral red about 50% of these bodies stained a deep pink, while the remainder were colorless. These particular cercariae when fixed in a fluid which contained ether yielded a large amount of a yellowish oil-like substance, leaving the furcae clear and transparent. From observations on the furcae of *B. papillo-*

sus I am of the opinion that these bodies are fatty in character and are due to an over-production of fat. When a cercaria is placed in distilled water, large vacuoles showing Brownian movements appear at irregular places on the furcae. These may indicate the position of pore openings. This response to distilled water was especially evident at the tips of the furcae.

While swimming, the body of the cercaria usually hangs downward with the furcae extended outward and upward in sigmoid curves. By alternate peristaltic extensions and contractions of the long furcae, the cercaria moves slowly and gracefully through quiet water. In a current the furcae are extended straight out from the axis of the body and held in that position as it is carried along. Badcock's (1875) description of the swimming of *B. polymorphus* as, "flying like eagles through the water with an upwards tendency," may well be applied to the present species.

When subjected to a sudden light shock the tail furcae quiver and then roll inward resembling a pair of coiled springs (Fig. 8). The cercaria remains in this condition for about three minutes before it recovers from the effect of the shock.

In marked contrast with the behavior of the tail furcae of *B. papillosus* during penetration, the furcae in the present species play an important rôle in the process. An analysis of a series of motion-pictures taken of this cercaria during the period of penetration shows that the tips of the furcae adhere to the fin of the fish. The penetration organ of the cercaria is then rammed against the host. When a small opening is made, the furcae by alternate contraction and extensions actively aid in forcing the body into the tissue of the host. When the body becomes securely anchored within the tissue, the furcae are pinched off at their base and the body draws away from the tail-stem, which then collapses. Peristaltic waves cause extension and contraction of the furca long after it is separated from the tail-stem. A cercaria was observed to complete its penetration within the space of two minutes.

One infected mussel contained a large number of cercariae that appeared to be in a stage of metamorphosis. The furcae were crumpled and the tail-stem was partly degenerated; the gut had contracted to a small size, resembling the gut of the adult and the anterior sucker formed by an invagination of the body wall had forced the penetration organ dorsad. The protrusible portion of the penetration organ had flattened out into the characteristic anterior surface of the adult. Thus the cercaria had taken on some of the characteristics of the adult or maritae without undergoing encystment. This advanced stage of development may have been due to the physical condition of the mussel, which had been in the aquarium all winter and also to the inability of the cercaria to emerge after being fully developed.

The cyst, formed within an hour after penetration is clear and unpigmented. It is slightly larger than the cyst of *B. papillosus*. Twenty un-

fixed cysts formed during the experimental infestation of small, large-mouthed bass measured in length from 0.081 to 0.1 mm., average 0.094; in width 0.056 to 0.081 mm., average 0.074. The distribution of the cysts in the tissues of the host is usually confined to the base of the fin rays although in small fish they may be also formed within the muscles.

Fins of small fish containing experimentally produced cysts were cut into small fragments and fed to an uninfected blue-gill, *Lepomis pallidus* (Mitchill) secured from the Third Sister Lake, a lake in which this parasite does not occur. One month later nine adult specimens of *B. elegans*, heavily laden with eggs were recovered from this fish.

***Bucephalus pusillus* (Stafford 1904)**

Synonym: *Gasterostomum pusillum* Stafford 1904

The adult of *B. pusillus* has been recorded only from the intestine of the wall-eyed pike, *Stizostedion vitreum* Mitchill. Stafford (1904) secured his fish from the markets of Montreal and Quebec. Aside from the measurements, his description, unaccompanied by drawings, was not detailed enough to adequately identify the species. The worm, which I describe and figure as *Bucephalus pusillus*, agrees with Stafford's measurements of specimens which he secured from *S. vitreum*. Cooper (1915) in describing a gasterostome which he identified as *G. pusillum* Stafford was in reality describing a new species, which I have redescribed in a previous paper as *Bucephalus papillosus* (Woodhead, 1929).

About 400 adult specimens of *B. pusillus* were obtained from the digestive tracts of 50 wall-eyed pike obtained from Saginaw Bay, Michigan, in November, 1926. Although about 50% of these worms contained eggs, no ripe eggs were secured due to the time consumed in isolating the adult from the excessive amount of cestodes and intestinal mucus. Adult gasterostomes were recovered from the intestine of a wall-eyed pike from Houghton Lake, Michigan, July 5, 1927. Out of a total of 156 specimens, 54 contained eggs. The eggs kept in culture dishes hatched at the end of one week. A large number of *B. pusillus* were found in several wall-eyed pike from Lake Winnipeg, Canada, in March, 1928. Out of 115 specimens isolated for study only 1 contained eggs.

Specific Diagnosis: Minute worms, 0.549 by 0.122 mm. Body cylindrical, with anterior sucker directed forward, having a dorsal and a ventral muscular lip. No papillae or other head appendages are present. Gut, 0.106 mm. in length, small, oval, dorsal to the pharynx (0.032 mm. in diameter) which is in the middle of the body. Cirrus pouch present on the left side. Testes two, averaging 0.060 mm. in diameter, one in front of the other.

Ovary 0.042 mm. in diameter between the gut and the anterior testes. Eggs oval, small and numerous.

The form of the body is cylindrical, when the worm is in a relaxed condition. In all of its structural characters except one, it agrees with the generic characters of *Bucephalus* as indicated in the description of *B. papillosus* (Woodhead, 1929). The one exception is in the structure of the anterior sucker. No papillae or fimbriae were observed in the many specimens examined. The anterior sucker is directed forward which is also a conspicuous difference between it and *B. papillosus* and *B. elegans* in both of which the anterior sucker is directed ventrad. The sucker is deeply concave; its dorsal lip is thick, its ventral lip thin.

The egg of *B. pusillus* is oval in outline, and has a well defined operculum. Measurement of 50 eggs showed a range of 0.028-0.032 mm. in length, average 0.031 and 0.018-0.021 mm. in width, average 0.020. Although the eggs of this form are the smallest of the three species described, they have proven valuable for study because of the abundance of the material and smaller amount of ciliary apparatus of the embryo. At least two flame cells were seen in one embryo still within the egg. This observation, however, has not been confirmed by further observations.

The miracidium (Fig. 21) of *B. pusillus* is very similar to that of *B. papillosus*. Its body is oval with four bristle-plates which are short and paddle-like, bearing a double row of long cilia. The anterior end terminates in a knob which is considerably more pronounced than in *B. papillosus*. There are no dorsal skeletal rods in this miracidium. Six swimming appendages are attached two dorsal, two ventral, two lateral, near the posterior end of the body. Each appendage has a single joint close to the body and bears long cilia on its outer surface. Locomotion is very rapid and is accomplished by the flapping of the bristle-plates and the vigorous kicking motions of the six posterior appendages.

The parthenitae, cercariae, and second intermediate host of this species are unknown.

SUMMARY

Bucephalus elegans sp. nov. is described and figured in all stages of its life history. Miracidium has ciliated appendages and four long cephalic bristle plates. Only one generation of sporocysts occurs. The cercariae are forked-tailed with very long furcae. Locomotion is slow and graceful. Cysts are formed in several species of fish. The life-history cycle is completed in the rock-bass.

The adult of *Bucephalus pusillus* (Stafford) is redescribed. The miracidium, reported for the first time has three pair of jointed appendages and four short cephalic plates.

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EXPLANATION OF PLATES

a	penetration spine
ao	anterior organ
bm	basement membrane
ca	branch of skeletal rod
ev	excretory vesicle
f	fimbria
gld	penetration gland duct
glp	pore opening
j	joint
n	notch on anterior surface
sr	skeletal rod

PLATE I

All figures concern *B. elegans*

- FIG. 1. Egg showing early cleavage stage of the miracidium embryo.
- FIG. 2. A later stage of the embryo showing the appearance of the bristle-plates and folded skeletal rods.
- FIG. 3. Showing a miracidium, subjected to slight pressure, emerging from the egg.
- FIG. 4. Dorsal view of miracidium, showing ciliary apparatus and penetration spine.
- FIG. 5. Semi-diagrammatic frontal view of the miracidium to show the arrangement of the bristle-plates.
- FIG. 6. Position assumed by the free-swimming cercaria, with the furcae extended.
- FIG. 7. The same as Fig. 6, but showing the furcae contracted.
- FIG. 8. A cercaria showing position of the furcae following either a light, or mechanical shock.
- FIG. 9. Frontal view to show the position assumed by the cercaria when floating with the current.
- FIG. 10. Partial view of a penetrating cercaria showing the shedding of furcae and tail-stem.
- FIG. 11. Diagrammatic sketch to show stages in the penetration process.
- FIG. 12. Ventral view of penetration organs of the cercaria, partly protruded.
- FIG. 13. Outline sketch of the digestive system of a cercaria, to show the gut in a reduced condition.
- FIG. 14. Ventral view of the mature cercaria, with details of structure, the distal parts of the furcae not shown.
- FIG. 15. Sketch of the excretory system in a flattened mature cercaria.

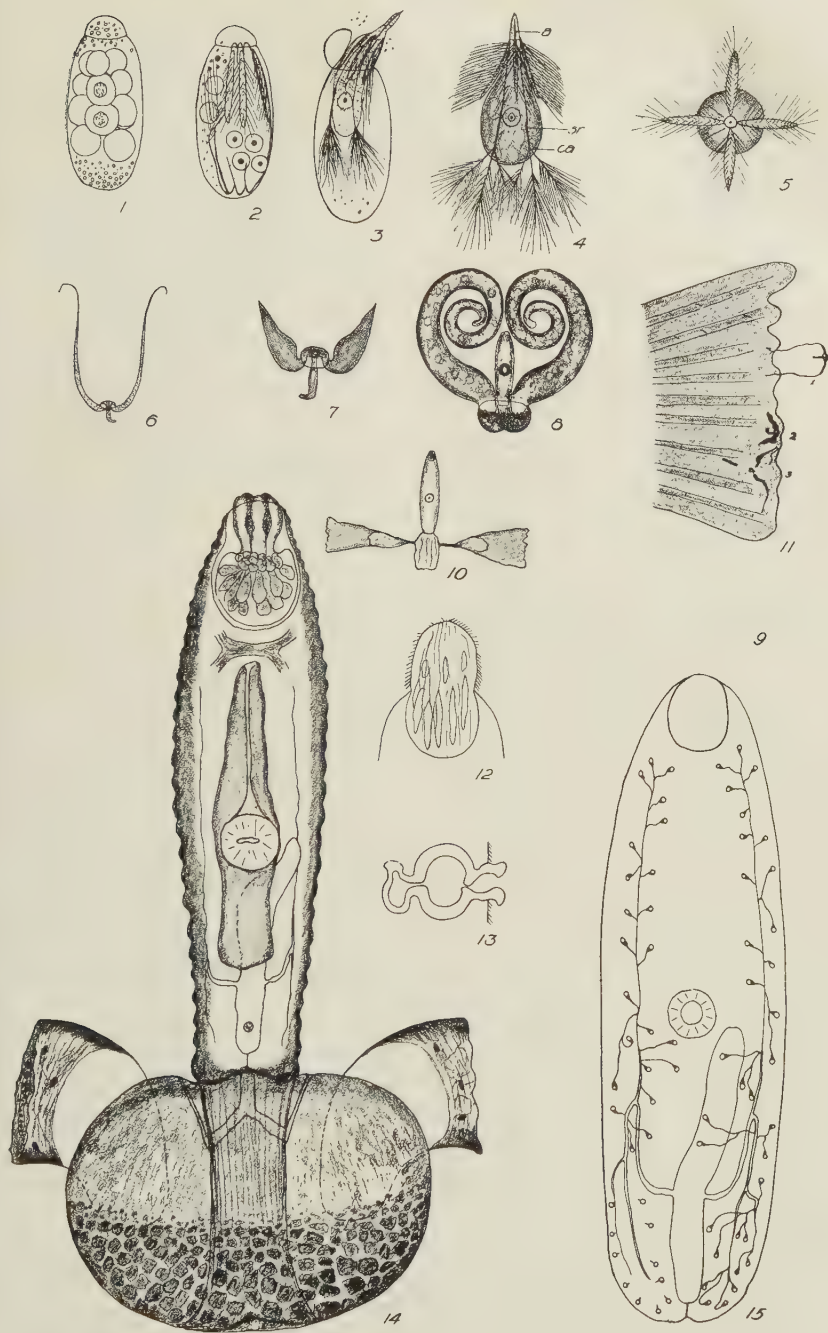


PLATE I

PLATE II

- FIG. 16. *B. elegans*: lateral view of a week-old cyst.
- FIG. 17. *B. elegans*: ventral view of head to show fimbriae partly extended, and the penetration gland ducts.
- FIG. 18. Lateral view of the same with the fimbriae fully extended.
- FIG. 19. *B. elegans*: frontal section of a living specimen through the region of the fully extended fimbriae.
- FIG. 20. *B. pusillus*: outline drawing of an egg.
- FIG. 21. *B. pusillus*: lateral view of the miracidium.
- FIG. 22. *B. elegans*: lateral view of adult with details of structure.
- FIG. 23. *B. elegans*: lateral and ventral views of dermal spines much enlarged.
- FIG. 24. *B. pusillus*: ventral view of the adult, with details of structure.

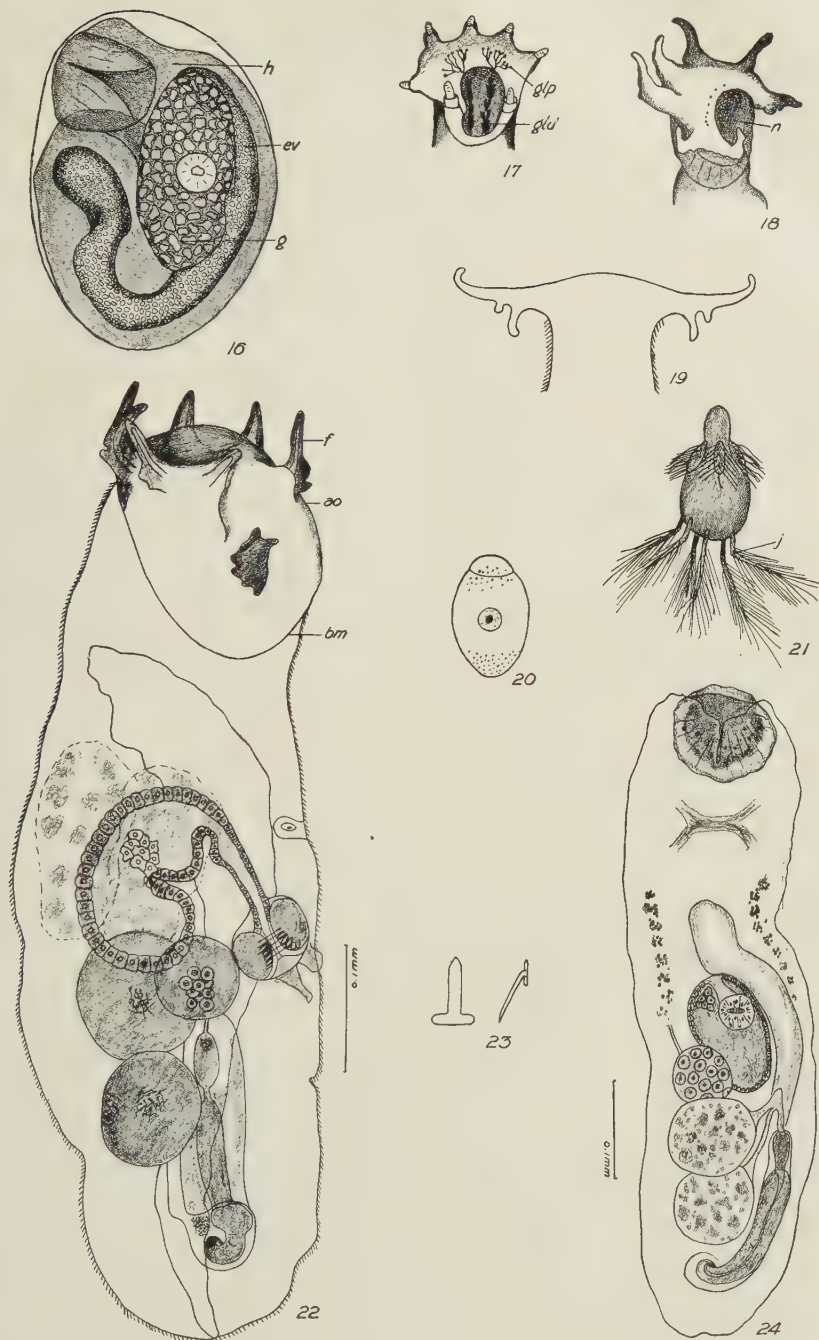


PLATE II

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THE GERM CELL CYCLE IN THE TREMATODE FAMILY BUCEPHALIDAE^{1, 2}

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While studying gasterostome material information was obtained which has a bearing on the recently revived subject of the "germ-cell cycle in trematodes." It is hoped that the observations herein presented will aid in the solution of this important problem. Instead of an examination of many forms "to find the universal method" of reproduction in trematodes the present paper is confined to one order, the Gasterostomata. The gasterostomes offer very favorable material for such a study due to the abundance of mother-sporocysts and cercariae.

MATERIALS AND TECHNIQUE

Three species of gasterostomes were used in the preparation of this paper: (1) *Bucephalus elegans* (Woodhead, 1930) from *Eurynia iris* and the rock-bass *Ambloplites rupestris*, (2) *B. papillosus* (Woodhead, 1929) from *Elliptio dilatatus* and the small-mouthed bass *Micropterus dolomieu*, (3) *B. pusillus* Cooper from *Anodonta grandis* and the wall-eyed pike *Stizostedion vitreum*. Many of the slides studied were made in preparation for "Life history studies on the Trematode family Bucephalidae, Nos. I and II," (Woodhead, 1929, 1930). New slides were also prepared and living material examined.

The abundance of sporocyst material made it possible to section entire clam bodies, infected in varying degree. Lightly infected material proved most satisfactory for a study of the various stages. From the standpoint of cytology the gasterostomes leave much to be desired. The cells are smaller than in some other forms that might have been selected. This disadvantage was partly offset by familiarity with the material.

Ends of living sporocysts examined with high-power yielded many clues

¹ Contribution from the Zoölogical Laboratory of the University of Michigan. This paper is No. III in the series "Life History Studies on the trematode Family Bucephalidae."

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that have been of great value in this problem. Living rediae were also studied. Toto mounts of both sporocysts and redia were prepared and stained with Delafield's haematoxylin. Parasitized clams were sectioned 5 microns. Serial sections of 3 microns were made of single redia, but proved unsatisfactory for a study of structure. Sections cut 3 microns were too thin, those of 5 microns were best, while the tens were worthless. A section cut 120 microns by mistake, when cleared but unstained, served as a toto mount showing sporocysts in position.

Fixatives used were Bouin's fluid, corrosive acetic, and Flemming's strong osmic. The latter resulted in confirming the presence of an abundance of fat, but also introduced a great amount of confusion. Stains used were: Heidenhain's iron haematoxylin without counterstain, Mallory's triple, and Flemming's modified triple. It was the use of this latter stain that made the following observations possible. The microscope used was a Leitz monobjective binocular, using 10x compensating, 15x and 20x oculars and a 1.8 mm. achromatic oil immersion as the highest objective.

PART I

THE REDIA OF THE GASTEROSTOMES

For many years the gasterostomes have been used as an example of a order of trematodes whose branching sporocyst gave rise parthenogenetically to cercariae, without an intermediate rediae generation. When studying longitudinal sections of mother-sporocysts, in an effort to find chromosomes or possibly even maturation stages, a structure was found which could be nothing else but a redia. On one slide overstained in Flemming's triple, safranin stained chromatin stood out sharply against a yellow background. This, in a structure assumed at the time to be a cercarial body, showed the chromatin in the form of crescents. This mass occupied the position in cercariae where one usually looks for the stomach and excretory bladder.

Nuclei with chromatin arranged in crescents indicated a stage in spermatogenesis. We were now faced with a most astounding condition, an *hermaphroditic redia*. Brooks (1930) has recently discarded "all polar bodies and maturation" in twenty species of trematodes and, therefore, the finding of a redia with definite gonads interferes with his proposed hypothesis of "polyembryony with germinal lineage."

With this newer knowledge of the presence of a redia, and having as a major clue the red crescents on a light yellow field, search for redia was made throughout all slides in my collection. Many redia were found. Why they had not been discovered before was probably due to two causes. (1) The size was so near that of the body of a cercaria that, unless differential staining had been employed, they could very easily be mistaken for cercariae. (2) Since the redia apparently does not leave the mother-sporocyst as is the usual

custom in other species, they were lost to view among all the developing cercariae. If they should be liberated along with the cercariae they could pass easily as a free-swimming rhabdocoele. Usually, however, the tips of the mother-sporocysts are not injured in dissection and the active redia, therefore, would not be liberated.

In the preparation of new slides some living material was examined to note the winter condition of the cercariae of *B. elegans*. In the dish was noted an active, heavily ciliated organism that was suspected of being a liberated redia. This specimen was lost and it became advisable to fix all the material. After staining in Ehrlich's acid haematoxylin, and mounting, a young branching mother-sporocyst (Fig. 11) was found free of cercariae but having many redia in all stages of development. This sporocyst had been destined to the point of being almost colorless, but served admirably for the study of toto mounts of the redia in position and definitely within the sporocyst.

Positions. Redia may be found in certain young, actively branching sporocysts, especially those in which no cercariae are present. If cercariae are present some mature rediae may be found among them. Using fine insect pins it is possible to remove the older rediae, sometimes singly, sometimes in groups of two or three fused together. Usually these older rediae will be bordering on a state of disintegration. In sections these rediae appear in corners of the lumen of the mother-sporocyst or attached to the walls and may be identified by the yellow color (orange G) of the testis and by the presence, in the mass, of numerous suckers. After becoming acquainted with the appearance of the free-swimming redia it is possible to recover it from a dissected mass of cercaria by means of a capillary mouth-pipette under the dissecting binocular.

Structure. The general shape of a young redia resembles a free swimming rhabdocoel turbellarian (Fig. 13). Long cilia cover the entire surface, being heavier in the anterior region. When swimming, the anterior end is sharply rounded, while the posterior end is drawn out into a short point. Two eye spots are present. The anterior half of the body is clear with several longitudinal muscle bands plainly visible. The posterior half of the body contains the reproductive organs. The testis occupies most of this region. Active spermatozoa were observed in localized areas in the large single testis.

Older rediae (Figs. 26, 27) apparently undergo a metamorphosis similar to that noticed in the older "final adult," where it was found that old age characters are developed, such as heavy spination and the extensible fimbriae (Woodhead, 1930). The older rediae possess a head "frill" extending above the anterior sucker. Spines appear arranged in a criss-cross pattern and the cilia are much reduced. A large anterior ventrally facing sucker is well developed. Dorsad to the sucker is a mass of "penetration" glands. The brain has two lateral branches to the two dorsally-placed eyes. No pigment could be discerned associated with cells of the eyes. From the wall in the vicinity

of the anterior sucker a suspensory mass of tissue supports the large centrally placed single testis. A ventral sucker is present. This sucker has all the appearance of the pharynx of the "final adult." Two protruding "lips" are well developed. Very careful observations were made to determine the presence of a digestive cavity in connection with the ventral sucker. No evidence was found to indicate that the ventral sucker was serving as a mouth and the only evidence of a gut was the presence of a few cells attached to the base of the pharynx.

The Testis. The testis which is central in position in the posterior half of the body, is oval in shape and composed of an outer mass of cells surrounding a central cavity. The cells are of two kinds (1) the germ cells and (2) secretory cells (prostate?). Extending posteriad is an extensible cirrus having a short median duct. In some sections the cirrus is prolonged and apparently capable of extension to the outside for cross-fertilization.

The Ovary. Very lightly stained mature rediae within the mother-sporocyst usually show a well-stained V-shaped mass of cells with the apex of the V extending to the posterior margin of the body. This structure is the female reproductive apparatus. The ovary is small, and is situated on the left side of the body. The ova are discharged anteriorly into the oviduct. The oviduct then turns posteriad and to the right. Laurer's canal enters at this first turn. The wall of the short oviduct is formed of characteristic cells, similar in appearance to the cells lining the oviducts of the "final adult." Near the posterior margin of the body the oviduct is joined by the vitelline duct. Laurer's canal from its union with the oviduct extends dorsad and then anteriorly opening on the dorsal surface opposite the ventral sucker. In one section of a redia, spermatozoa were collected in a mass in a cavity near the dorsal-opening and also were scattered throughout the length of the canal.

Yolk glands. The nutritive complex is very well developed, appearing as paired lateral, stroma-like masses of vitelline follicles extending from the region of the anterior sucker, to the posterior region. There the two lateral ducts unite on the right side of the body and discharge vitelline cells through a short duct into the lumen of the mother-sporocyst. This short common vitelline duct appears as the right side of the V-shaped structure previously mentioned. The yolk-cells are large and undergo many important changes. When finally discharged as small vitelline cells the chromatin is arranged in patches near the periphery of the nucleus. It is this type of cell that Brooks (1930) mistakes for germ-cells labeling them as "antecedent germ-cells" and in which he found "no evidence of polar bodies or other maturation phenomena." Some of the yolk cells when discharged along with the fertilized egg apparently attach themselves to the egg. This process has introduced many erroneous interpretations into the literature. By some authors the yolk material is seen as "micromeres," others as extrusion of nuclear chromatin. The exact steps in

the nutritive cycle needs to be studied as a separate problem. I believe, however, that this material has been elaborated by the redia to serve as food for the germ ball and early larval stages of cercariae.

The Excretory System has not been determined due to scarcity of living material.

It is evident from the structures outlined above that we have in this redia an intermediate generation that has evolved to a stage that has many of the characters of the "final adult."

The discovery of the redia introduced two other very important problems. (1) To understand the process of spermatogenesis that apparently takes place in the structure suspected of being a testis, it became necessary to study the details of the process as exemplified in the "final adult." The result of this study is briefly described in part III of this paper. (2) With a redia present in the life cycle it also became necessary to determine its origin. Tennent (1906) had described the production of "cercarial" germ-balls by two methods. Search was made through the sporocyst for germ-cells and embryos that could produce redia. The observations are recorded in part II of this paper.

PART II

THE SPOROCYST GENERATION

Living sporocysts of *Bucephalus pusillus* from *Anodonta grandis*, are remarkable for their ability to swing from side to side and expand and contract. This movement is restricted to the extreme tips of the branching sporocysts. The expanded region, packed with cercariae shows no movement. In a living sporocyst (Fig. 1) the cercariae, actively squirming, were prevented by a large mass of cells (t) from penetrating to the end of the short, restricted terminal region. When the specimen was about to become dry the cercariae forced their way into the restricted lumen, which then exploded leaving a small residue in the form of a strip of cells. Later study of sections showed this strip of cells to be the ovary.

After sectioning some of this same material search for a longitudinal section that would compare with the tip observed in the living condition proved decidedly interesting (Fig. 3). The large mass of cells, near the beginning of the restricted lumen, evidently was a testis. Spermatogenesis in the final adult of *B. elegans*, *B. papillosus*, and *B. pusillus* had to be worked out in detail (Part III of this paper) in order to understand the evidence observed in this mass of cells and thereby prove this structure clearly to be a testis. I fully realize the significance of such an interpretation. If this were proven to be a testis, then the whole reproductive cycle of trematodes would need to be carefully investigated. It is hard to believe that the Gasterostomata stands alone and far removed from other trematode groups in this matter of reproduction.

That this mass of cells is a functional testis is indicated briefly as fol-

lows: At A in Fig. 3, spermatids with crescent-shaped chromatin are clearly visible, under oil-immersion and 10x compensating oculars. This condition had been observed in the newly found testis of the redia and was also found in stages of spermatogenesis in the final adult. At B, are cells lightly stained (lack of chromatin specificity at this stage) showing transformation of spermatids. Mature spermatozoa, deeply stained are conspicuously evident at C. Stages A, B, C all occur in one follicle. Other stages of spermatogenesis occur in the mass. Also at D cells having the appearance of "fixed" spermatozoa are found in a tubular-like space. At E they appear to be in the lumen of the mother-sporocyst but to be collected at the opening of a tube (Laurer's canal?) in which other spermatozoa appear at F and a larger collection at G.

The ovary was located in the form of "stroma" (Fig. 3, O) attached to the lateral wall near the tip and extending downward to the testis. That this structure was an ovary was indicated by the presence of cells undergoing maturation. Polar bodies were being formed.

For confirmation of this arrangement of organs and the presence of male and female gonads more material was prepared. The bodies of seven small *Eurythia iris* infected with *B. elegans* were fixed with strong Flemming's and stained in Flemming's modified triple stain. Longitudinal sections of sporocyst-ends were found showing the ovary within a "plug-like" structure continuous with the inner lining of the mother-sporocyst and extending downward into the lumen. Due probably to shrinkage in fixation the "plug" in this material was not in contact with the side-walls of the mother-sporocyst. I believe it fills the lumen in living material and thereby would more closely resemble the arrangement in *B. pusillus* previously described, which had been fixed in Bouin's fluid. This "plug" was composed of tissues of several types; (a) reproductive, (b) nutritive, (c) supporting. At the distal end yolk glands were discharging vitelline cells into the lumen. Germ cells were undergoing maturation in the central portion of the plug. A duct was apparently present leading into the central mass from a point on the side adjacent to the yolk glands. This duct may indicate the early origin of Laurer's canal (a duct leading to the ovary for the entrance of spermatozoa) although, as yet, no evidence is at hand, other than morphological, to support this concept.

At a short distance below the plug, the lumen of the mother-sporocyst was almost completely obstructed by a large mass of cells in which spermatozoa and prostate (?) secretions were being formed. This structure in *B. elegans* therefore confirmed the findings in *B. pusillus*. Moreover sections showed what is interpreted to be fertilization (Figs. 8, 9, 10). Also the cleavage stages following were abundant. The fertilized ovum does not have the power of movement and as no apparatus is present to project them into the lumen, they remain near the place of origin and develop into germ-balls. Cleavage is apparently nearly equal in the early stages. As cleavage progresses the

embryo becomes encapsulated in the "supporting" tissue which then grows rapidly downward through the lumen, thus preventing crowding at the place of origin. As the ovary is producing its store of ova and the supporting tissue grows downward the tip grows away from the used testis. Apparently new gonads are formed at intervals on the inner wall. Tennent (1906, p. 650) described two methods of producing cercariae in *Bucephalus haimeanus*. "Besides the method of forming the germ-cells which is above described, another often occurs. This takes place towards the close of the first method. In this second method, instead of the scattered production of germ-cells, there is a localization of production; a definite portion of the wall of the sporocyst, usually at one end of the tube, grows into the lumen as a blunt process (Fig. 22), which functions as a special organ for the production of germs. In these processes the germ-cells, which are there differentiated, undergo their stages of segmentation and reach the condition of a germ-ball of considerable size, when they burst the tissue enclosing them and come to lie in the lumen of the tube where their further development takes place." When the redia was found his description became understandable. His "first" method is *not* germ cells from the walls but are *fertilized ova* from the redia, shed into the lumen and coming to lie next to the wall. His "second" method is the formation of rediae by the mother sporocyst. Brooks (1930, p. 322) interpreted this paragraph from Tennent to mean: "The present writer interprets the original cells to be antecedent germ cells and the 'blunt process' to be a germ-mass lying adjacent to the wall. 'The bursting of the tissue enclosing them' is a graphic description of the process of dissociation. Tennent's further description of the cells after they have been discharged into the body cavity fits splendidly the ex-components of the writer's forms except for the production of three polar bodies. This is interpreted to be the formation of the first three micromeres."

PART III

SPERMATOGENESIS

A. In the Final Adult. The finding of suspected spermatogenesis in the redia and mother-sporocyst made it imperative to have a better knowledge of the details of the process. As there never has been any question concerning the presence of testes in the "final adult" stage, sections of *B. elegans* and *B. pusillus* were therefore carefully studied. This material had been stained in Flemming's triple and should present a picture comparable to the sections of sporocysts and rediae. The process of spermatogenesis as observed in *B. elegans* may be briefly outlined as follows: (a) In a resting condition the primary spermatogonia are located within the wall of the testis and there undergo mitotic multiplication. The nuclei of the germ cells are larger than the nuclei of adjacent somatic cells and stain more deeply. Twelve chromosomes are present. (b) When maturation is about to begin, the primary spermatogonia migrate

to the inner surface of the testis. Growth follows and the cell protrudes into the central cavity. At this period the nucleus is surrounded by cytoplasm giving a clear picture of a cell. (c) The germ-cell next becomes detached from the wall and is free in the cavity. (d) The cytoplasm then is drawn out at opposite ends of the nucleus. This condition is an important clue in the recognition of the germ cells. Brooks (1930) in figures 34, 39, 40, 42, and 45 shows this condition in other species, but does not interpret the cells as germ cells. (e) The germ cell then divides into two cells of equal size that are best labeled *secondary* spermatogonia. The old cell membrane serves as an enclosing membrane. The cytoplasm is not evident as in the previous stage. (f) A second division results in the formation of four cells, *tertiary* spermatogonia*, of equal size; each cell growing to the size of the primary spermatogonia that was first free in the cavity. (g) The chromatin now appears in the form of conspicuous granules. The four nuclei have grown larger. (h) The chromatin next loses its conspicuous appearance. The nuclei are now many times the original size and the enclosing membrane is very thin. (i) Within each nucleus the chromatin assumes the form of a spiral ribbon. (j) The margin of this ribbon becomes scalloped. (k) Chromosomes with connecting strands are now evident (early tetrads). (l) The group of four tertiary spermatogonia then fuses with neighboring groups. (m) The nuclei become much smaller and the cytoplasm of each cell is again plainly visible. The nuclei collect at the periphery of the fused mass of cells, the cytoplasm being massed in the center. The twelve chromosomes emerge as six well defined tetrads. (n) The tetrads move apart and secondary spermatocysts are formed. (o) The spermatids are next formed and the chromatin which at first is in the center begins to collect at the outer margin of the cell. (p) The chromatin next condenses into a crescent-shaped deeply staining mass with the convex surface directed outward from the cell. (q) The next step takes place apparently after a period of arrested development (in the crescent form). The crescent-shaped chromatin mass straightens and loses its specificity for stains. (r) The head when formed again takes the stain deeply and the cytoplasm elongates into a tail. (s) Mature spermatozoa are now ready to migrate from the cytoplasmic mass. The fused masses or rosettes may have been formed originally from many tertiary spermatogonia and yield numbers of spermatozoa but always in multiples of sixteen.

B. In the Redia. The germ cells of the redia at the beginning of the maturation process, are attached by stalk-like projections to the walls of the testis. The process of maturation is hard to follow due to the small size of the cells. Sufficient stages have been found, however, to warrant the belief that the process is similar to that observed in the final adult. The most conspicu-

* NOTE: I am indebted to Dr. H. W. Stunkard for suggestions which helped to clear up this complicated process.

ous stages are: (a) Cells with opposite placed protoplasmic projections. (b) Cells undergoing growth and nuclear changes. (c) A group of four cells, interpreted as being tertiary spermatogonia. (d) Tetrads in early stage. (e) Spermatids with chromatin arranged in crescents. This stage is very common. (f) Spermatozoa in rosettes. (g) Living spermatozoa.

The maturation process appears to go on for some time after the rest of the body of the redia has begun to degenerate. Whether or not this continued maturation has any significance I am unable to say at present although it resembles the condition in the final adult where spermatozoa are formed for some time after the ovary ceases to function. Living spermatozoa observed in a free-swimming redia mitigates against any argument that the testis is non-functional. It is also very interesting to observe the well defined gonads in the gasterostome redia and compare this with the arguments for and against their existence in rediae of other species.

C. In the Mother Sporocyst. The stages in maturation that have been observed are: (a) the groups of secondary and tertiary spermatogonia, (b) the spireme, (c) the tetrads, (d) the spermatids with the chromatin in crescent form, (e) transformation with loss of stain specificity, (f) the rosettes of mature spermatozoa, (g) living spermatozoa, (h) fertilization (in sections). This last stage is interpreted as conclusive proof of sexual reproduction in the mother-sporocyst generation.

DISCUSSION

Brooks (1930) has outlined the history of the germ cell cycle in Trematodes about as follows:

I. Metagenesis = Alternation of an ovular with an anovular generation. Supported by: Steenstrup (1842), Moulinie (1856), Pagenstecher (1857), Wagener (1866), Biehringer (1884), Balfour (1885).

II. Heterogeny = Alternation of a bi-sexual with a parthenogenetic generation. Grobben (1882), Reuss (1903), Haswell (1903), Tennent (1904), Carey (1909), Faust (1917). All but Grobben report finding polar bodies.

III. Paedogenesis = Reproduction by the larva and not by the adult. Introduced by von Baer (1866). Supported in theory by Ssinitzin (1911) and Sewell (1922).

IV. Extended metamorphosis = Spread out developmental phases. Balfour (1885), Leuckart (1886), Looss (1892).

V. Germinal Lineage = Germ cells extending through this peculiar life history. Leuckart laid the foundation, modified and added to by Thomas (1883), Schwarze (1886), Coe (1896), Dollfus (1919) who also mentions polyembryony.

VI. Germinal Lineage through Polyembryony = idea of Brooks: "A substitute hypothesis is offered interpreting the life history as one in which

the germinal lineage passes through successive larval stages in which polyembryony features as a mode of multiplication and in which precocious cleavage is an activating factor."

The gasterostome sporocyst can not be considered "just an empty sac." In any study of this branching sporocyst one must consider the mass as a dendritic colony which has budded profusely. The ends or tips of the branches are to be considered the active portion of a mature worm with a full complex of functional organs. The portion of the branch used as a brood-pouch for the cercariae represents a region incapable of movement due to the large numbers of offspring within it.

That we have three "adults" I believe is certain. The three adults are mature and not larval. All three have a well defined anterior sucker with mucin and penetration glands. The reproductive and nutritive systems in each are well developed. I see no "larval" structure in either the first, second or third adults and therefore no "paedogenesis." The gasterostome is therefore polymorphic having three generations (Plate V), each generation having homologous stages; 1st the fertilized egg, 2nd the free-swimming larvae (miracidium, pro-redia and cercariae), 3rd the adult with a pupal (cyst) stage retained or evolved only in the last generation.

Relatively little change other than position has occurred in the reproductive organs in the three generations of a life cycle. The rediae advance is shown by the increased amount of yolk material. The reproductive complex in the third or final adult has been modified by increasing the length of the oviduct to provide a uterus for retention of eggs until the embryo is well developed. This is a response to the need for carrying the species over the hazard of a free stage in the water.

SUMMARY

1. An intermediate generation, the redia, new to the literature of the gasterostomes is described and figured.
2. Spermatogenesis is described for the mother-sporocyst, the redia and the final adult.
3. Laurer's canal appears in the redia as a long tube, containing sperm-cells, thereby supporting the theory of its use in fertilization.
4. The ovary is localized in the mother-sporocyst and redia.
5. Each generation begins with a fertilized egg.
6. Reduction and fertilization occurs in each generation.
7. There is no parthenogenesis or metagenesis.
8. Reproduction is bi-sexual in all stages of this polymorphic species.

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EXPLANATION OF PLATES

b	lateral bud
bp	blunt process or plug
cl	closed portion of lumen
cr	cytoplasmic residue
cs	cirrus
cy	cytoplasm of cell
e	eye spot
er	embryonic redia
gld	glandular area
gp	genital pore
l	lumen
lc	Laurer's canal
nc	nurse or vitelline cell
no	nucleolus
o	ovary
od	oviduct
ph	pharynx
r	redia
sr	sperm receptacle
st	supporting tissue
t	testis
v	vitellarian follicle
w	wall of mother-sporocyst

PLATE XVII

Mother sporocyst of *B. pusillum*.

FIG. 1. Distal end of a sporocyst, drawn from living specimen.

FIG. 2. The same specimen after cercariae had forced their way into the "closed-lumen," showing the cellular residue remaining.

FIG. 3. A longitudinal section of the distal end showing testis, ovary and glandular area.

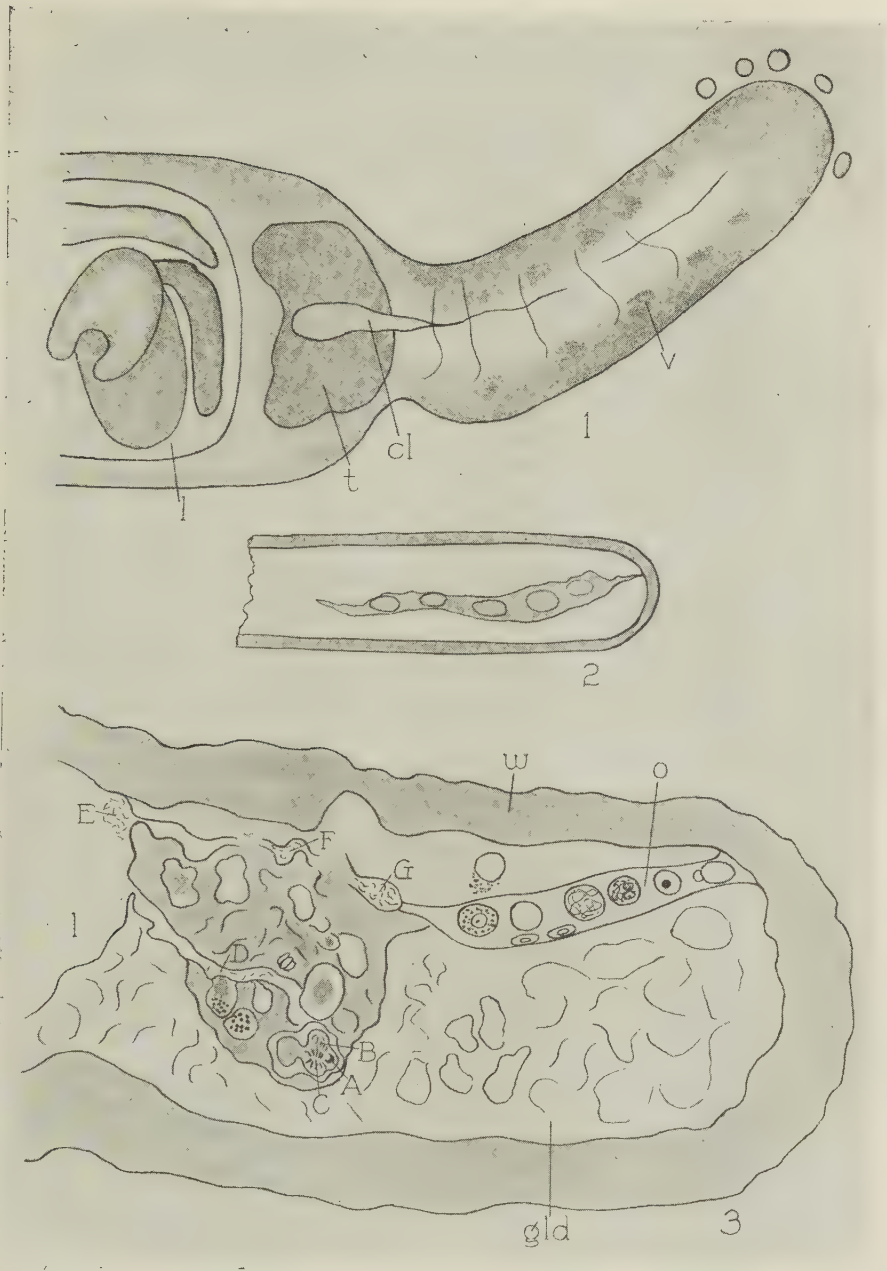


PLATE XVIII

Mother sporocyst of *B. elegans*.

- FIG. 4. Syncytium containing four tertiary spermatogonia.
FIG. 5. Syncytium containing two secondary spermatogonia.
FIG. 6. Formation of tetrads (early).
FIG. 7. A primary spermatogonia preparing to divide into secondary spermatogonia.
FIG. 8, 9, 10. Three stages in fertilization showing approach and fusion of the pro-nuclei.
FIG. 11. An actively reproducing branch of the mother sporocyst showing redia in the lumen. (Drawn with the aid of a camera lucida).
FIG. 12. A longitudinal section of the distal end of a branch showing relation between the blunt process and the testis.

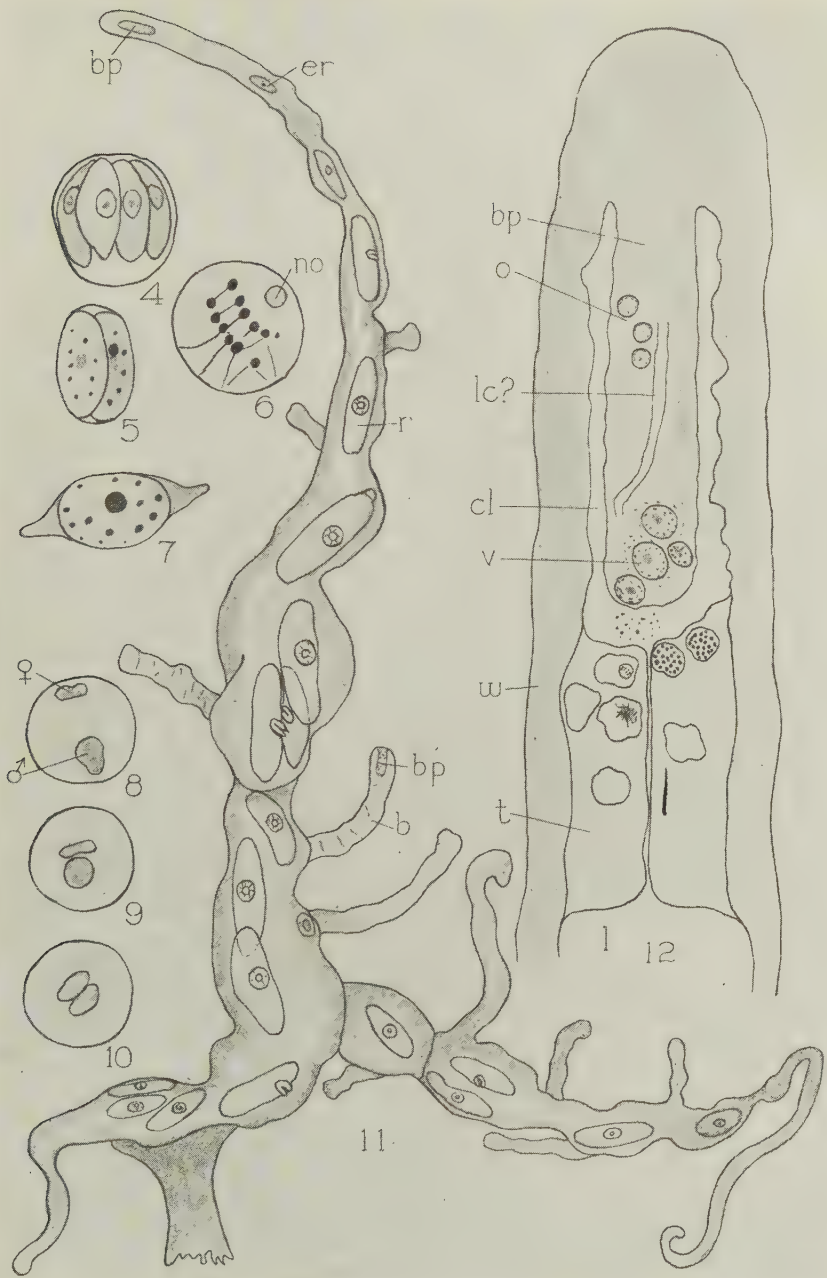


PLATE XIX

Redial generation of *B. elegans*.

- FIG. 13. Young redia, drawn from life.
FIG. 14. A primary spermatogonia.
FIG. 15. The four tertiary spermatogonia.
FIG. 16. Early tetrad formation.
FIG. 17. Spermatid, showing crescent-shaped mass of chromatin.
FIG. 18. Spermatid before transformation.
FIG. 19. Spermatid beginning to transform.
FIG. 20. Head of mature spermatozoa.
FIG. 21. Fertilized egg of the redia, drawn from life. Showing cytoplasm about the nucleus.
FIG. 22. Yolk cell as shed by the redia before its breaking up into vitelline cells.
FIG. 23. A yolk-cell as it appears in an early stage of development.
FIG. 24. A newly shed fertilized egg of the redia, surrounded by single vitelline cells.
FIG. 25. An egg cell as it appears soon after the absorption of yolk materials.
FIG. 26. Redia, ventral view.
FIG. 27. Redia, side view.
FIG. 28. Portion of "supporting" stroma, elongated from the blunt-process of the mother-sporocyst, showing two stages in development of a redia.
FIG. 29. Very young, ciliated redia, dissected from mother-sporocyst, drawn from life.

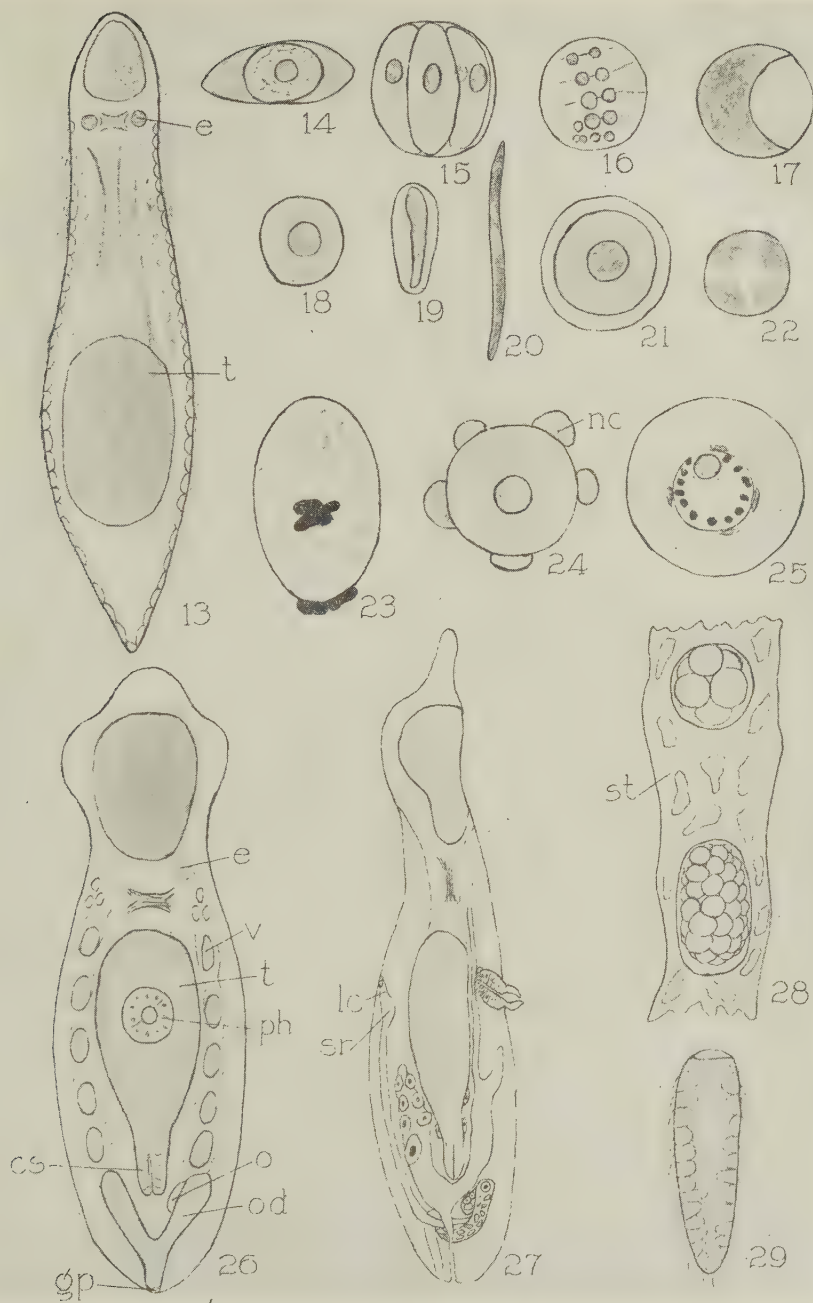
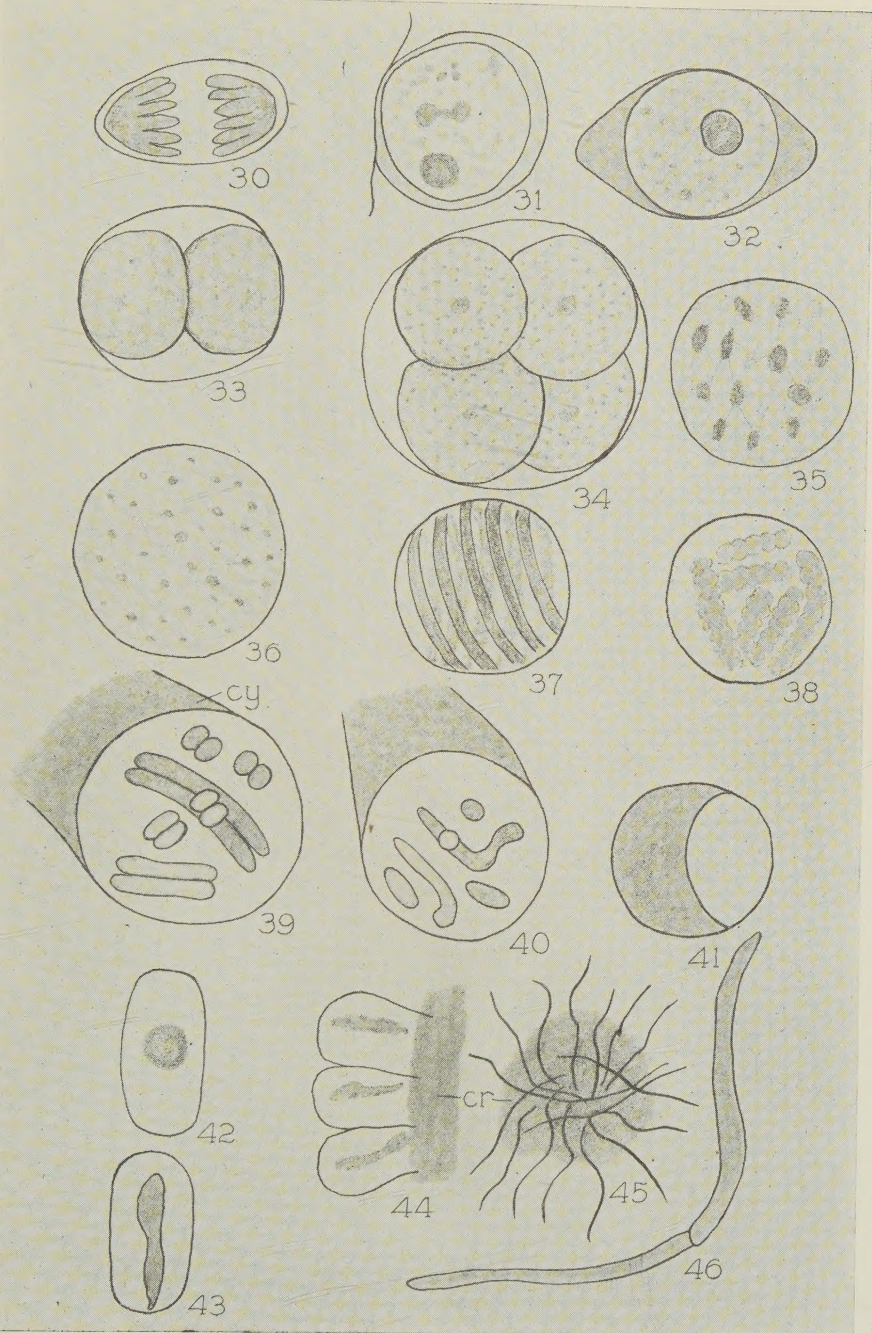


PLATE XX

Spermatogenesis in final adult of *B. elegans*.

- FIG. 30. Telophase of spermatogonia within the wall of the testis.
FIG. 31. Primary spermatogonium attached to inner wall of testis.
FIG. 32. Primary spermatogonium, free from wall, showing cytoplasmic prolongations.
FIG. 33. Secondary spermatogonia.
FIG. 34. Tertiary spermatogonia.
FIG. 35, 36, 37, 38. Nuclear changes preceding synapsis.
FIG. 39. Primary spermatocyte.
FIG. 40. Secondary spermatocyte.
FIG. 41. Spermatid.
FIG. 42, 43. Changes during metamorphosis of spermatid.
FIG. 44. Edge of fused mass showing clear cells on the margin.
FIG. 45. Fused mass showing emerging spermatozoa and cytoplasmic residue.
FIG. 46. Head of mature spermatozoa.



ADULT GENERATIONS

